CHAPTER

2 Hematology

contents

I. FUNDAMENTAL HEMATOLOGY PRINCIPLES

A. Blood Composition

1. **Whole blood** includes erythrocytes, leukocytes, platelets, and plasma. When a specimen is centrifuged, leukocytes and platelets make up the **buffy coat** (small white layer of cells lying between the packed red blood cells and the plasma).
2. **Plasma** is the liquid portion of unclotted blood. **Serum** is the fluid that remains after coagulation has occurred and a clot has formed.
   a. Plasma is composed of 90% water and contains proteins, enzymes, hormones, lipids, and salts.
   b. Plasma normally appears hazy and pale yellow (contains all coagulation proteins), and serum normally appears clear and straw colored (lacks fibrinogen group coagulation proteins).

B. Basic Hematology Terminology

a-	without
-blast	youngest/nucleated
-chromic	colored
-cyte	cell
dys-	abnormal
-emia	in the blood
ferro-	iron
hyper-	increased
hypo-	decreased
iso-	equal
macro-	large
mega-	very large/huge
micro-	small
myelo-	marrow
normo-	normal
-oid	like
-osis	increased
pan-	all
-penia	decreased
-plasia	formation
-poiesis	cell production
poly-	many
pro-	before
thrombo-	clot

C. Formed Elements and Sizes

Formed Element	Size
1. Thrombocytes (platelets)	2–4 μm
2. Erythrocytes (RBCs)	6–8 μm
3. Normal lymphocytes	6–9 μm
4. Reactive lymphocytes	10–22 μm
5. Basophils	10–15 μm
6. Segmented neutrophils	10–15 μm
7. Band neutrophils	10–15 μm
8. Eosinophils	12–16 μm
9. Monocytes	12–20 μm

D. Basic Homeostasis

1. **Homeostasis** is the body's tendency to move toward physiological stability. *In vitro* testing of blood and other body fluids must replicate exact environmental body conditions. These conditions should include the following:
   a. **Osmotic concentration** is the body/cellular water concentration, composed of 0.85% sodium chloride. This normal osmotic concentration is termed **isotonic.** In a **hypotonic** solution (greater amount of H_2O in relationship to lesser amount of solutes), water enters the cell; the cell **swells** and may lyse. In a **hypertonic** solution (lesser amount of H_2O in relationship to greater amount of solutes), water leaves the cell; the cell may **crenate.**
   b. **pH reference range:** Venous blood range 7.36–7.41; arterial blood range 7.38–7.44
   c. **Temperature:** Normal body temperature is 37.0°C. Blood specimens should be analyzed as soon as possible to prevent cellular breakdown (refer to individual tests for specimen collection requirements, stability times, and storage temperature).

E. RBC Indices

1. **MCV (mean corpuscular volume):** Reference range (SI/conventional units) is 80–100 femtoliters (fL), and it is an indicator of the average/mean

volume of erythrocytes (RBCs). Calculate using the hematocrit (Hct) and RBC count:

$$\text{MCV (fL)} = \frac{\text{Hct (\%)} \times 10}{\text{RBC count } (\times 10^{12}/\text{L})}$$

a. **Increased** in megaloblastic anemia, hemolytic anemia with reticulocytosis, liver disease, and normal newborn
b. **Decreased** in iron deficiency anemia, thalassemia, sideroblastic anemia, and lead poisoning

2. **MCH (mean corpuscular hemoglobin):** Reference range (SI/conventional units) is 26–34 picograms (pg), and it is an indicator of the average weight of hemoglobin in individual RBCs. Calculate using the hemoglobin (Hgb) and RBC count:

$$\text{MCH (pg)} = \frac{\text{Hemoglobin (g/dL)} \times 10}{\text{RBC count } (\times 10^{12}/\text{L})}$$

a. **Increased** in macrocytic anemia
b. **Decreased** in microcytic, hypochromic anemia

3. **MCHC (mean corpuscular hemoglobin concentration):** Reference range (conventional units) is 32–37 g/dL (SI units 320–370 g/L), and it is a measure of the average concentration of hemoglobin in grams per deciliter. Calculate using the hemoglobin and hematocrit values:

$$\text{MCHC (g/dL)} = \frac{\text{Hemoglobin (g/dL)}}{\text{Hct}} \times 100$$

a. 32–37 g/dL MCHC indicates **normochromic** RBCs.
b. Lesser than (<) 32 g/dL MCHC indicates **hypochromic** RBCs, which is seen in iron deficiency and thalassemia.
c. Greater than (>) 37 g/dL MCHC indicates a possible error in RBC or hemoglobin measurement, or the presence of **spherocytes.**

F. Other RBC Parameters

1. **RDW (RBC distribution width):** Reference range (conventional units) is 11.5–14.5%.
   a. Determined from the RBC histogram
   b. Increased proportional to the degree of anisocytosis (variation in size); coefficient of variation of the mean corpuscular volume
   c. High RDW: Seen post-transfusion, post-treatment (e.g., iron, B_{12}, or folic acid therapy), idiopathic sideroblastic anemia, in the presence of two concurrent deficiencies (iron and folic acid deficiencies)

2. **Hct (Hematocrit):** Reference range for males (conventional units) is 41–53% (SI units 0.41–0.53 L/L). Reference range for females (conventional units) is 36–46% (SI units 0.36–0.46 L/L). Reference range for hematocrit is age and sex dependent. Hematocrit is the percentage of RBCs in a given volume of whole blood.
   a. Spun **microhematocrit** is the reference manual method.
   b. The buffy coat layer of leukocytes and platelets, not included in the measurement, can be seen between plasma (upper) and RBC (lower) layers.
   c. Hematocrit is calculated by many automated cell counters using the MCV and RBC count:

$$\text{Hct }\% = \frac{\text{MCV (fL)} \times \text{RBC count } (\times 10^{12}/\text{L})}{10}$$

3. **Hgb (Hemoglobin):** Reference range for males (conventional units) is 13.5–17.5 g/dL (SI units 135–175 g/L). Reference range for females (conventional units) is 12.0–16.0 g/dL (SI units 120–160 g/L). Reference range for hemoglobin is age and sex dependent.

G. Platelets

1. **PLT (Platelets):** Reference range (SI units) is 150–450 $\times 10^9$/L (conventional units 150,000–450,000/μL).
2. **MPV (mean platelet volume):** Reference range (SI/conventional units) is 6.8–10.2 fL. MPV is analogous to the MCV for erythrocytes.

H. Relative and Absolute Blood Cell Counts

1. **Relative count** is the amount of a cell type in relation to other blood components. **Relative lymphocytosis** is an increase in the **percentage** of lymphocytes; this is frequently associated with neutropenia. In **relative polycythemia,** RBCs appear increased due to a decreased plasma volume.
2. **Absolute count** is the actual number of each cell type without respect to other blood components. **Absolute lymphocytosis** is a true increase in the **number** of lymphocytes. **Absolute polycythemia** is a true increase in **red cell mass.**

I. Hematology Stains

1. **Nonvital (dead cell) polychrome stain (Romanowsky)**
   a. Most commonly used routine peripheral blood smear stain is **Wright's stain.**
   b. **Wright's stain** contains **methylene blue,** a basic dye, which stains acidic cellular components (DNA and RNA) blue, and **eosin,** an acidic dye, which stains basic components (hemoglobin and eosinophilic cytoplasmic granules) red-orange.

c. **Methanol fixative** is used in the staining process to fix the cells to the slide.
d. Staining does not begin until a **phosphate buffer** (pH between 6.4 and 6.8) is added.
e. Causes of **RBCs too red** and **WBC nuclei poorly stained:** Buffer or stain below pH 6.4, excess buffer, decreased staining time, increased washing time, thin smear, expired stains
f. Causes of **RBCs and WBC nuclei too blue:** Buffer or stain above pH 6.8, too little buffer, increased staining time, poor washing, thick smear, increased protein, heparinized blood sample
g. Examples of polychrome stains include: **Wright, Giemsa,** Leishman, Jenner, May-Grünwald, and various combinations of them

2. **Nonvital monochrome stain**
   a. Stains specific cellular components
   b. **Prussian blue** stain is an example.
      1) Contains potassium ferrocyanide, HCl, and a safranin counterstain
      2) Used to visualize **iron granules** in RBCs (siderotic iron granules), histiocytes, and urine epithelial cells
3. **Supravital** (living cell) **monochrome stain**
   a. Used to stain specific cellular components
   b. No fixatives are used in the staining process.
   c. Includes:
      1) **New methylene blue** used to precipitate RNA in **reticulocytes;** measure of bone marrow **erythropoiesis**
      2) **Neutral red** with **brilliant cresyl green** as a counterstain is used to visualize **Heinz bodies;** clinical disorders associated with Heinz bodies include **G6PD deficiency** and other **unstable hemoglobin** disorders.

II. HEMATOPOIESIS

A. Hematopoiesis

1. Production and differentiation of blood cells
2. Blood cell production, maturation, and death occur in organs of the **reticuloendo-thelial system** (RES).
   a. RES includes bone marrow, spleen, liver, thymus, lymph nodes.
   b. RES functions in **hematopoiesis, phagocytosis,** and **immune defense.**
3. **Intrauterine hematopoiesis** includes three phases:
   a. **Mesoblastic (yolk sac) phase** begins at ~19 days gestation. The **yolk sac** is located outside the developing embryo. The first cell to be produced is a **primitive nucleated erythroblast.** This cell produces embryonic hemoglobins: **Portland, Gower I and Gower II.** Alpha-globin chain production begins at this phase and continues throughout life.
   b. **Hepatic** (liver) **phase** begins at 6 weeks gestation with production of mainly **red blood cells,** but also granulocytes, monocytes, and

megakaryocytes. Alpha- and gamma-globin chain production predominates forming **Hgb F;** detectable Hgb A and A_2 are also present.

c. **Myeloid/medullary phase** begins around the fifth month of gestation, with the **bone marrow** producing mainly **granulocytes.** The M:E (myeloid:erythroid) ratio approaches the adult level of 3:1. Alpha- and gamma-globin chain production predominates at birth, forming **Hgb F;** Hgb A and A_2 are also present. Hgb A will not predominate until 6 months of age when the gamma-beta globin chain switch is complete.

4. At birth, the bone marrow is very cellular with mainly **red marrow,** indicating very active blood cell production. Red marrow is gradually replaced by inactive **yellow marrow** composed of fat. Under physiological stress, yellow marrow may revert to active red marrow.

B. Pediatric and Adult Hematopoiesis

1. **Bone marrow**
   a. Newborn: 80–90% of bone marrow is active red marrow.
   b. Young adult (age 20): 60% of bone marrow is active. Hematopoiesis is confined to the proximal ends of large flat bones, pelvis, and sternum.
   c. Older adult (age 55): 40% of bone marrow is active; 60% is fat.
   d. **Cellularity** is the ratio of marrow cells to fat (red marrow/yellow marrow) and is described in adults as:
      1) **Normocellular**— Marrow has 30–70% hematopoietic cells.
      2) **Hypercellular/hyperplastic**—Marrow has >70% hematopoietic cells.
      3) **Hypocellular/hypoplastic**—Marrow has <30% hematopoietic cells.
      4) **Aplastic**—Marrow has few or no hematopoietic cells.
   e. **M:E (myeloid:erythroid) ratio** is the ratio of granulocytes and their precursors to nucleated erythroid precursors. A normal ratio is between **3:1** and **4:1.** Granulocytes are more numerous because of their short survival (1–2 days) as compared to erythrocytes with a 120-day life span. Lymphocytes and monocytes are excluded from the M:E ratio.
   f. Stem cell theory
      1) Hematopoiesis involves the production of **pluripotential stem cells** that develop into **committed progenitor cells (lymphoid or myeloid)** and finally mature blood cells.
         a) **Progenitor cells**
            i) **Lymphoid:** Differentiate into either **B or T lymphocytes** in response to cytokines/lymphokines/interleukins/CSFs/growth factors
            ii) **Myeloid:** Gives rise to the multipotential progenitor **CFU-GEMM (colony-forming-unit-granulocyte-erythrocyte-macrophage-megakaryocyte),** which will differentiate into **committed progenitor cells** and finally **mature blood cells** in

response to cytokines/interleukins/colony stimulating factors/growth factors:

Committed Progenitor Cell		Growth Factors/ Interleukins	Mature Cell
CFU-MEG		Thrombopoietin, GM-CSF	Thrombocytes
CFU-GM	CFU-M	GM-CSF, M-CSF, IL-3	Monocytes
CFU-GM	CFU-G	GM-CSF, G-CSF, IL-3	Neutrophils
BFU-E	CFU-E	Erythropoietin, GM-CSF, IL-3	Erythrocytes
CFU-Eo		GM-CSF, IL-3, IL-5	Eosinophils
CFU-Ba		IL-3, IL-4	Basophils

2. **Lymphoid tissue**
   a. **Primary lymphoid tissue**
      1) Bone marrow: Site of pre–B cell differentiation
      2) Thymus: Site of pre–T cell differentiation
      3) This is antigen-**independent** lymphopoiesis.
   b. **Secondary lymphoid tissue**
      1) B and T lymphocytes enter the blood and populate secondary lymphoid tissue, where antigen contact occurs.
      2) Includes lymph nodes, spleen, gut-associated tissue (Peyer's patches)
      3) Antigen-**dependent** lymphopoiesis depends on antigenic stimulation of T and B lymphocytes.

C. Introduction to Leukocytes

1. Classified as **phagocytes** (granulocytes, monocytes) or **immunocytes** (lymphocytes, plasma cells, and monocytes)
2. **WBC reference range** (SI units) is $4.0–11.0 \times 10^9$/L (conventional units $4.0–11.0 \times 10^3/\mu L$).
3. **Granulocytes** include neutrophils, eosinophils, and basophils.
4. **Neutrophils** are the first to reach the tissues and **phagocytize** (destroy) bacteria. In the process, they die.
5. **Monocytes** differentiate into **macrophages,** and as such they work in the tissues to **phagocytize** foreign bodies. They arrive at the site of inflammation after neutrophils and do not die in the process.
6. **T lymphocytes** provide **cellular immunity.** They represent 80% of lymphocytes in the blood. When activated, they proliferate and produce **cytokines/interleukins.**

7. **B lymphocytes** develop into **plasma cells** in the tissue and produce **antibodies** needed for **humoral immunity.** B lymphocytes represent 20% of the lymphocytes in the blood.
8. **NK** (natural killer) **lymphocytes** destroy tumor cells and cells infected with viruses. They are also known as **large granular lymphocytes** (LGLs).
9. **Eosinophils** modulate the allergic response caused by basophil degranulation.
10. **Basophils** mediate immediate hypersensitivity reactions (type I, anaphylactic).
11. **CD markers** are surface proteins expressed by **specific cell lines** at different maturation stages. As a cell matures, some markers vanish and new ones appear. More than 200 CD markers have been identified. Commonly used markers include the following:

CD2, CD3	Lymphoid, pan T cells
CD4	Helper/inducer T cells
CD8	Suppressor/cytotoxic T cells
CD13	Pan myeloid
CD11c, CD14	Monocytes
CD19, CD20	Lymphoid, pan B cells
CD33	Pan myeloid cells
CD34	Stem cell marker (lymphoid and myeloid precursor)
CD16, CD56	NK cells

D. Medullary versus Extramedullary Hematopoiesis

1. **Medullary hematopoiesis:** Blood cell production **within** the bone marrow
   a. Begins in the fifth month of gestation and continues throughout life
2. **Extramedullary hematopoiesis:** Blood cell production **outside** the bone marrow
   a. Occurs when the bone marrow cannot meet body requirements
   b. Occurs mainly in the liver and spleen; hepatomegaly and/or splenomegaly often accompany this

E. Basic Cell Morphology

1. **Nucleus**
   a. Contains **chromatin** composed of **DNA** and proteins
   b. Contains **nucleoli** rich in **RNA**

2. **Cytoplasm**
   a. **Golgi complex** forms lysosomes.
   b. **Lysosomes** contain hydrolytic enzymes that participate in phagocytosis.
   c. **Ribosomes** assemble amino acids into protein.
   d. **Mitochondria** furnish the cell with energy (ATP).
   e. **Endoplasmic reticulum** is a system of interconnected tubes for protein and lipid transport.

F. General Cell Maturation Characteristics for Leukocytes

Immature Cells	Mature Cells
Cell is large	Cell becomes smaller
Nucleoli present	Nucleoli absent
Chromatin fine and delicate	Chromatin coarse and clumped
Nucleus round	Nucleus round, lobulated, or segmented
Cytoplasm dark blue (rich in RNA)	Cytoplasm light blue (less RNA)
High N:C ratio	Low N:C ratio

III. GRANULOCYTES

A. Basic Review

1. The myeloid progenitor cell gives rise to a committed progenitor cell that is acted on by growth factors to form granulocytes.

B. Maturation and Morphology of Immature Granulocytes

1. **Myeloblast:** Earliest recognizable granulocyte precursor
   a. 14–20 μm
   b. N:C ratio 7:1–4:1
   c. Round/oval nucleus with fine reddish-purple staining chromatin
   d. 2–5 nucleoli
   e. Dark blue cytoplasm
   f. **No cytoplasmic granules**
   g. 1% of the nucleated cells in the bone marrow
2. **Promyelocyte**
   a. 15–21 μm
   b. N:C ratio 3:1
   c. Round/oval nucleus with slightly coarsening chromatin

   d. 1–3 nucleoli
   e. Dark blue cytoplasm
   f. Cytoplasm has large, **nonspecific/primary granules** containing **myeloperoxidase.**
   g. 2–5% of the nucleated cells in the bone marrow
3. **Myelocyte:** First stage where granulocyte types can be differentiated into eosinophils, basophils, and neutrophils
   a. 12–18 μm
   b. N:C ratio 2:1
   c. Round nucleus with coarse chromatin
   d. Early myelocytes may have visible nucleoli.
   e. Light blue to light pink cytoplasm
   f. Prominent **golgi apparatus—clear area** located in the cytoplasm next to the nucleus
   g. Cytoplasm has **specific/secondary granules** that contain **hydrolytic enzymes,** including **alkaline phosphatase** and **lysozyme.**
   h. **Nonspecific/primary granules** are present and may still stain.
   i. Last stage capable of cell division
   j. **Neutrophilic myelocyte** makes up 13% of the nucleated cells in the bone marrow.
4. **Metamyelocyte**
   a. 10–18 μm
   b. N:C ratio 1.5:1
   c. Nucleus is indented in a **kidney bean** shape and has coarse, clumped chromatin.
   d. Nuclear indent is **less than** half the width of a hypothetical round nucleus.
   e. Cytoplasm is pink and filled with pale blue to pink **specific/secondary granules.**
   f. **Nonspecific/primary granules** are present but usually do not stain.
   g. **Neutrophilic metamyelocyte** makes up 16% of the nucleated cells in the bone marrow.
5. **Band neutrophil**
   a. 10–15 μm
   b. N:C ratio 1:2
   c. Nucleus is "C" or "S"-shaped with coarse, clumped chromatin **lacking segmentation.**
   d. Nuclear indent is **greater than** half the width of a hypothetical round nucleus.
   e. Cytoplasm is pink and filled with pale blue to pink **specific/secondary granules.**
   f. **Nonspecific/primary granules** are present but usually don't stain.
   g. **Band neutrophil** makes up 12% of the nucleated cells in the bone marrow, and 0–5% of peripheral white blood cells (WBCs).

h. Stored in the bone marrow and released when there is an increased demand for neutrophils

C. Morphology of Mature Granulocytes

1. **Segmented neutrophil** (Referred to as: seg, polymorphonuclear cell (PMN), and poly)
   a. 10–15 μm
   b. N:C ratio 1:3
   c. Nucleus has coarse, clumped chromatin with **3–5 lobes** connected by thin filaments.
   d. Cytoplasm is pink and filled with small, pale blue to pink **specific/secondary granules.**
   e. **Nonspecific/primary granules** are present but usually do not stain unless in response to infection or growth factor.
   f. **Segmented neutrophil** makes up 12% of the nucleated cells in the bone marrow, and 50–80% of peripheral WBCs.
2. **Eosinophil**
   a. Recognizable maturation stages include the eosinophilic myelocyte, eosinophilic metamyelocyte, eosinophilic band, and eosinophil (segmented form).
   b. Eosinophils are 12–16 μm.
   c. Nucleus is usually bilobed.
   d. Cytoplasm contains large, **bright red-orange,** secondary granules that contain **enzymes** and **proteins.**
   e. Eosinophils make up less than 1% of the nucleated cells in the bone marrow and 5% of peripheral WBCs
3. **Basophil**
   a. Recognizable maturation stages include the basophilic myelocyte, basophilic metamyelocyte, basophilic band, and basophil (segmented form).
   b. Basophils are 10–15 μm.
   c. Cytoplasm contains large, **purple-black,** secondary granules that contain **heparin** and **histamine.**
   d. Granules may be numerous and obscure the nucleus, or they may "**wash out**" in staining (because the granules are water soluble) and leave empty areas in the cytoplasm.
   e. Basophils make up less than 0.1% of the nucleated cells in both the bone marrow and peripheral blood

D. Granulocyte Function

1. **Neutrophils**
   a. Blasts, promyelocytes, and myelocytes are in the bone marrow **mitotic pool** 3–6 days, and that is where they **divide.**

b. Metamyelocytes, bands, and segmented neutrophils are in the bone marrow **post-mitotic pool** about 6 days, and that is where they **mature.**
c. Released into circulation when mature or when needed
d. Total blood granulocyte pool
    1) Contains 50% **circulating granulocyte pool** (mainly neutrophils) that is measured when a WBC count is performed
    2) Contains 50% **marginating granulocyte pool** (mainly neutrophils) that adheres to vessel walls
    3) There is a rapid and free exchange of neutrophils between the circulating granulocyte pool and marginating granulocyte pool.
    4) Neutrophils **diapedese** into the **tissues** from the marginating pool in response to **antigenic stimulation.**
    5) **Chemotactic factors** attract the neutrophil to the site of inflammation; include **complement, bacterial products, injured tissue, hemostatic components.**
    6) **Opsonins** such as **IgG** and complement component **C3b** help neutrophils recognize a substance as foreign.
    7) **Phagocytosis** involves neutrophil attachment to the foreign object, formation of a vacuole around it, and neutrophilic degranulation to release lytic enzymes (**respiratory burst**) in an effort to kill the organism.
    8) Neutrophils are sensitive to the oxidants they secrete and are destroyed in the process.
e. Blood and tissue cells in the body undergo **cell death** through **necrosis** or **apoptosis.**
    1) **Necrosis** is induced by **extracellular** forces such as lethal chemical, biological, or physical events. The blood cell is "killed."
    2) **Apoptosis** is "**programmed cell death**" due to extracellular or **intracellular** processes that depend on a signal.

2. **Visible response to infection by neutrophils (toxic changes)**
    a. Toxic changes are associated with **bacterial infection** or **growth factor therapy.** Any combination of these changes may be seen in some but not necessarily all of the neutrophils.
    b. **Toxic granulation** is prominent granulation due to persistent staining of **primary granules.** Neutrophilic cytoplasm normally contains only visible, small, **secondary granules.**
    c. **Toxic vacuolation:** Colorless areas in the cytoplasm that indicate phagocytosis and degranulation have occurred
    d. **Döhle bodies:** Small oval inclusions **(RNA)** located in the cytoplasm stain light blue
    e. **Shift to the left** refers to an increased number of myelocytes, metamyelocytes, and/or bands in the peripheral blood. It is associated with either increased or decreased WBC counts.

1) **Regenerative shift to the left** is an appropriate bone marrow response to increased demand for neutrophils. It is seen in infection or in other physiological or pathological conditions requiring neutrophils.
    a) WBC count above the reference range
    b) Most common type of left shift
2) **Degenerative shift to the left** is seen after an overwhelming infection in which bone marrow production cannot keep up with increased need for neutrophils.
    a) Associated with a poor prognosis
    b) WBC count below the reference range

3. **Eosinophils**
    a. In the blood only a few hours before seeking a tissue site such as nasal passages, skin, or urinary tract
    b. They can degranulate like neutrophils. They express Fc receptors for **IgE,** which is a response to **parasitic infections.**
    c. They release substances that can neutralize products released by basophils and mast cells; eosinophils modulate the **allergic** response.
4. **Basophils**
    a. In the blood only a few hours before migrating to the site of inflammation in the tissues
    b. They express membrane receptors for **IgE.** Once activated, degranulation releases histamine. This initiates the classic signs **of immediate hypersensitivity reactions (Type I).**
    c. Basophils release a chemotactic factor that attracts eosinophils to the site.

E. Nonmalignant Granulocytic Disorders

1. **Shift/physiologic/pseudoneutrophilia**
    a. Redistribution of the blood pools causes a short-term **increase** in the total WBC count and in the absolute number of neutrophils in the **circulating granulocyte pool.**
    b. Caused by exercise, stress, pain, pregnancy
    c. It is not a response to tissue damage. The **total blood granulocyte pool** in the body has not changed. The bone marrow has not released immature neutrophils. There are no toxic changes, and there is **no shift to the left.**
2. **Pathologic neutrophilia**
    a. Neutrophils leave the circulating pool, enter the marginating pool, and then move to the tissues in response to tissue damage.
    b. Bone marrow reserves are released into the blood to replenish the circulating pool. The **WBC count can increase up to 50.0 $\times$ 10^9/L,** and there is a **shift to the left** with toxic changes to the neutrophils.
    c. Bone marrow **increases production** of neutrophils to replenish reserves.
    d. Occurs in response to **bacterial** and other infections, **tissue destruction,** drugs or toxins, **growth factor,** etc.

3. **Neutrophilic leukemoid reaction** (NLR)
   a. Blood picture mimics that seen in **chronic myelogenous leukemia.**
   b. **Benign,** extreme response to a specific agent or stimulus
   c. The **WBC count can increase to between 50.0 and 100.0 $\times$ 10^9/L,** and there is a **shift to the left** with toxic changes to the neutrophils.
4. **Leukoerythroblastic reaction**
   a. Presence of **immature leukocytes** and **immature (nucleated) erythrocytes** in the blood
   b. Occurs in marrow replacement disorders, such as **myelofibrosis**
5. **Neutropenia**
   a. **Decrease** in absolute number of **neutrophils; risk of infection** increases as neutropenia worsens
   b. Due to **bone marrow production defects:**
      1) Chronic or severe infection depletes available neutrophil reserves. **Use exceeds bone marrow production.**
      2) **Hypersplenism** causes neutrophils to be removed from circulation.
      3) Bone marrow **injury** (aplastic anemia), bone marrow **infiltration** (leukemia, myelodysplastic syndromes, or metastatic cancer), bone marrow **suppression** by chemicals or drugs (chemotherapy)
      4) **DNA synthesis** defects due to vitamin B_{12} or folate deficiency
      5) Many **viral infections** are associated with neutropenia.
6. **Eosinophilia**
   a. **Increase** in the absolute number of **eosinophils**
   b. Associated with:
      1) **Parasitic infections, allergic reactions, chronic inflammation**
      2) Chronic myelogenous leukemia, including early maturation stages, Hodgkin disease, tumors
7. **Eosinopenia**
   a. **Decrease** in the absolute number of **eosinophils**
   b. Seen in **acute inflammation** and inflammatory reactions that cause release of glucocorticosteroids and epinephrine
8. **Basophilia**
   a. **Increase** in the absolute number of **basophils**
   b. Associated with:
      1) **Type I hypersensitivity reactions**
      2) **Chronic myelogenous leukemia,** including **early maturation stages,** polycythemia vera
      3) **Relative transient basophilia** can be seen in patients on hematopoietic growth factors.
9. **Basopenia**
   a. **Decrease** in the absolute number of **basophils** associated with inflammatory states and following immunologic reactions
   b. Difficult to diagnose because of their normally low reference range

10. **Functional disorders of neutrophils**
    a. **Chronic granulomatous disease (CGD)**
        1) Both **sex-linked** and autosomal recessive inheritance with the ratio of affected males to females being 6:1
        2) Morphologically normal, but functionally abnormal because of enzyme deficiency that results in an inability to degranulate, which causes inhibited bactericidal function
        3) Fatal early in life
    b. **Chédiak-Higashi syndrome**
        1) Autosomal recessive disorder causes large, gray-green, peroxidase positive granules in the cytoplasm of leukocytes; abnormal fusion of primary and secondary neutrophilic granules
        2) Both **morphologically** and **functionally abnormal** leukocytes; WBCs unable to degranulate and kill invading bacteria
        3) Patients will present with photophobia and skin hypopigmentation.
        4) Fatal early in life
11. **Nuclear abnormalities of neutrophils**
    a. **Hypersegmentation** characterized by **5 or more lobes** in the **neutrophil;** associated with **megaloblastic anemia** due to vitamin B_{12} or folic acid deficiencies
    b. **Hyposegmentation** refers to a tendency in **neutrophils** to have **1 or 2 lobes;** may indicate an anomaly or a shift to the left.
        1) **Pelger-Huët anomaly**
            a) **Autosomal dominant** inheritance
            b) Nucleus is hyperclumped, and it does not mature past the two-lobed stage.
            c) Nucleus dumbbell- or peanut shaped; referred to as "pince-nez"
            d) Morphologically abnormal, but **functionally normal**
            e) Must **differentiate from a shift to the left** associated with an infection (toxic changes); infection requires treatment but Pelger-Huët anomaly (no toxic changes) does not.
        2) **Pseudo Pelger-Huët**
            a) **Acquired** abnormality associated with **myeloproliferative disorders** and **myelodysplastic syndromes;** can also be drug induced
            b) **Nucleus** is usually **round** instead of the dumbbell shape that is seen in the anomaly.
            c) Frequently accompanied by **hypogranulation**
12. **Inherited cytoplasmic anomalies**
    a. **May-Hegglin anomaly**
        1) **Autosomal dominant** inheritance
        2) Large, crystalline, **Döhle-like inclusions** in the cytoplasm of neutrophils on Wright's stain; gray-blue and spindle (cigar) shaped

3) Morphologically abnormal, but **functionally normal**
4) **Giant platelets, thrombocytopenia,** and clinical bleeding are also associated with this anomaly.

b. **Alder-Reilly anomaly**
1) **Autosomal recessive** inheritance
2) **Large azurophilic** granules appear in cytoplasm of **all or only one cell line.** Granules contain degraded mucopolysaccharides due to an enzyme defect.
3) Morphologically abnormal, but **functionally normal**
4) Must differentiate from **toxic granulation** present in **neutrophils only** in infectious conditions

IV. MONOCYTES AND MACROPHAGES

A. Basic Review

1. The **myeloid progenitor cell** gives rise to a committed progenitor cell, **CFU-GM** (colony-forming-unit-granulocyte-macrophage), that is acted on by growth factors (GM-CSF) and interleukins (ILs) to form **monocytes.** Monocytes form in the bone marrow, pass through the peripheral blood, and then migrate into the tissues **(macro-phages),** where they fight infection. Macrophages are named according to their location in the body.
   a. **Monocytes** —peripheral blood
   b. **Kupffer cells**—liver
   c. **Microglial cells**—central nervous system
   d. **Osteoclasts**—bone
   e. **Langerhans' cells**—skin
   f. **Alveolar cells**—lung

B. Maturation and Morphology of Monocytes

1. **Monoblast:** Earliest recognizable monocyte precursor
   a. 12–18 μm; N:C ratio 4:1
   b. Round/oval eccentric nucleus with fine chromatin; 1–2 nucleoli
   c. Dark blue cytoplasm; may have a gray tint; **no cytoplasmic granules**
2. **Promonocyte**
   a. 12–20 μm; N:C ratio 3:1
   b. Irregularly shaped, indented nucleus with fine chromatin; 0–1 nucleoli
   c. Blue to gray cytoplasm; fine azurophilic granules
3. **Monocyte**
   a. 12–20 μm
   b. Horseshoe- or kidney-bean-shaped nucleus, often with "brainlike" convolutions
   c. Fine, lacy chromatin
   d. Blue-gray cytoplasm; may have pseudopods and vacuoles

e. Many fine azurophilic granules give the appearance of "ground glass."
f. Transitional cell because it migrates into the tissue and becomes a fixed or free macrophage

4. **Macrophage:** "Tissue monocyte"
   a. 15–80 μm
   b. Indented, elongated, or egg-shaped nucleus with fine chromatin
   c. Blue-gray cytoplasm with many vacuoles and coarse azurophilic granules; may contain ingested material

C. Monocyte Characteristics

1. Granules are lysosomes that contain hydrolytic enzymes, including peroxidase and acid phosphatase.
2. Highly motile cell that marginates against vessel walls and into the tissues
3. Reference range is 2–10% in peripheral blood.

D. Monocyte/Macrophage Function

1. Play a major role in **initiating** and **regulating** the **immune response**
2. They process ingested material and also **process antigenic information,** which is relayed to the **T-helper (CD4)** lymphocyte. The T-helper lymphocyte coordinates the immune response to foreign antigens.
3. They arrive at the site of inflammation after neutrophils. Unlike neutrophils, the phagocytic process does not kill the monocyte.
4. Very efficient **phagocytic** cells with receptors for IgG or complement-coated organisms
5. Known as **"scavenger cells"** because of their ability to ingest foreign material
   a. Blood monocytes ingest **antigen-antibody complexes** and **activated clotting factors,** limiting the coagulation response.
   b. Splenic macrophages remove **old/damaged RBCs** and conserve iron for recycling.
   c. Liver macrophages remove **fibrin degradation products.**
   d. Bone marrow macrophages remove abnormal RBCs, ingest bare **megakaryocyte nuclei** or **extruded RBC nuclei,** and store and supply **iron** for hemoglobin synthesis.
6. Monocytes secrete **cytokines/interleukins** and **tumor necrosis factor.**

E. Nonmalignant Monocytic Disorders

1. **Monocytosis**
   a. **Increase** in the absolute number of **monocytes** associated with:
      1) Recovery stage from acute bacterial infections and recovery following marrow suppression by drugs
      2) Tuberculosis, syphilis, subacute bacterial endocarditis
      3) Autoimmune disorders (systemic lupus erythematosus, rheumatoid arthritis)

2. **Lipid storage disorders**
   a. **Gaucher disease** is the most common lipid storage disorder and has an autosomal recessive inheritance pattern. A deficiency in glucocerebrosidase causes glucocerebroside to accumulate in macrophages of the bone marrow, spleen, and liver, with **Gaucher cells** more commonly seen in the bone marrow.
   b. **Niemann-Pick disease** has an autosomal recessive inheritance pattern. A deficiency in sphingomyelinase causes sphingomyelin to accumulate in macrophages in multiple organs and bone marrow, where **Niemann-Pick cells** can be seen.
   c. **Sea-blue histiocytosis** is caused by an unknown deficiency. Sea-blue macrophages are found in the spleen and bone marrow.
   d. Others include Tay-Sachs and Fabry diseases
3. **Monocytopenia**
   a. **Decrease** in the absolute number of **monocytes**
   b. Associated with stem cell disorders such as **aplastic anemia**

V. LYMPHOCYTES AND PLASMA CELLS

A. Basic Review

1. The pluripotential stem cell gives rise to the **lymphoid progenitor cell** that is acted on by colony stimulating factors/interleukins/cytokines to form **B** and **T lymphocytes. Pre–B lymphocytes** differentiate in the **bone marrow,** and **pre–T lymphocytes** differentiate in the **thymus** through **antigen-independent lymphopoiesis.**
2. Bone marrow and thymus are **primary lymphoid tissues.**
3. B- and T cells enter the blood and populate the **secondary lymphoid tissues** (lymph nodes, spleen, and Peyer's patches in the intestine), where antigen contact occurs.

B. Maturation and Morphology of Lymphocytes

1. **Lymphoblast:** Earliest recognizable lymphocyte precursor
   a. 10–18 μm; N:C ratio 4:1
   b. Round/oval eccentric nucleus with fine chromatin; 1 or more nucleoli
   c. Dark blue cytoplasm; **no cytoplasmic granules**
2. **Prolymphocyte**
   a. 9–18 μm; N:C ratio 3:1
   b. Round or indented nucleus with coarsening chromatin; 0–1 nucleoli
   c. Basophilic cytoplasm; **no cytoplasmic granules**
3. **Lymphocyte**
   a. 7–18 μm
   b. Round, oval, or slightly indented nucleus; condensed chromatin
   c. Scant to moderate amount of blue cytoplasm; **few azurophilic granules**

4. **Reactive lymphocytes** have become activated as part of the immune response. Associated with **lymphocytosis** and can show the following characteristics:
   a. Generally, larger cell with increased amount of dark blue cytoplasm (RNA)
   b. Fine chromatin pattern with nucleoli
   c. Irregular shape to the nucleus
   d. Irregular shape to the cytoplasm (tags, sharp ridges); indented by red cells

C. T Lymphocytes (T cells)

1. Become immunocompetent in the secondary lymphoid tissue; **dependent on antigenic stimulation**
   a. Acquire specific receptors for antigens
   b. Make up **80%** of the peripheral blood lymphocytes
2. They are identified by membrane markers **CD2, CD3,** and others. The markers appear, disappear, and then reappear throughout cell development.
3. **T lymphocyte function**
   a. T cells provide **cellular immunity.** They are responsible for graft rejections and lysis of neoplastic cells, and they attack/destroy viral and fungal organisms.
   b. Obtain antigenic information from monocytes; this information is passed to other T cells and B cells
   c. Regulate humoral response by helping antigens activate B cells
   d. End products of activation are **cytokines/lymphokines/interleukins**
4. Three **T cell subsets** are involved in the immune response and are differentiated by cluster designation **(CD)** markers.
   a. **T helper/inducer cell (T-h, T_4)**
      1) Identified by **CD4** membrane marker
      2) Promotes activation of B cells by antigens
   b. **T suppressor cell (T-s, T_8)**
      1) Identified by **CD8** membrane marker
      2) Suppresses activation of B cells by antigens
   c. **Cytotoxic T cell (T-c, T_8)**
      1) Identified by **CD8** membrane marker
      2) Functions in viral infections and organ rejections
   d. The normal **T_4:T_8 ratio** in circulating blood is **2:1.** This ratio must be maintained for proper immune response. It is used to monitor HIV patients. T helper (CD4) cells are destroyed by the HIV virus, which decreases the ratio as the infection spreads.

D. B Lymphocytes (B cells)

1. Become immunocompetent in the secondary lymphoid tissue; **dependent on antigenic stimulation.**
   a. Acquire specific receptors for antigens
   b. Make up **20%** of the peripheral blood lymphocytes
2. Identified by membrane markers **CD19, CD20,** and others

3. **B lymphocyte function**
   a. Contact with foreign antigens stimulates B lymphocytes to become **reactive lymphocytes,** with the characteristic morphology associated with reactivity.
   b. Reactive lymphocytes transform into **immunoblasts,** and then **plasma cells** that produce **antibodies** to provide **humoral immunity.**
   c. **Plasma cells**
      1) End stage of B lymphocyte; dominant in lymph nodes; not normally seen in circulation
      2) 10–20 μm
      3) Abundant blue cytoplasm with prominent **perinuclear (golgi) zone**
      4) **Eccentric nucleus** with a very coarse, clumped chromatin pattern
      5) Make up less than 4% of nucleated cells in the bone marrow

E. Natural Killer (NK)/Large Granular Lymphocytes (LGLs)

1. Large cells with low N:C ratio, large cytoplasmic granules, and pale blue cytoplasm
2. Lack B cell or T cell membrane markers; are **CD16 and CD56 positive**
3. Responsible for surveillance of cells for surface alterations such as tumor cells or cells infected with viruses
4. Activated by IL-2 to express nonspecific cytotoxic functions
5. Attack antigens with attached IgG; called **antibody-dependent cytotoxic cells**

F. Nonmalignant Lymphocytosis Associated with Viral Infections

1. **Infectious mononucleosis**
   a. **Epstein-Barr** virus (EBV) infects **B lymphocytes.**
   b. Common in the 14–24 age group with symptoms ranging from malaise and fever to pharyngitis, lymphadenopathy, and splenomegaly
   c. Transmitted through nasopharyngeal secretions
   d. Lymphocytes usually >50% of the WBCs, with 20% being **reactive T lymphocytes** attacking affected B lymphocytes
   e. **Positive heterophile antibody** test
2. **Cytomegalovirus (CMV)**
   a. Symptoms similar to infectious mononucleosis
   b. Transmission is by blood transfusions and saliva exchange.
   c. 90% of lymphocytes can be reactive.
   d. Negative heterophile antibody test
   e. Transfused blood products are often tested for CMV.
3. **Infectious lymphocytosis**
   a. Associated with **adenovirus** and **coxsackie A** virus
   b. Contagious disease mostly affecting young children
   c. After a 12- to 21-day incubation period, symptoms appear and include vomiting, fever, rash, diarrhea, and possible CNS involvement.
   d. Lymphocytosis with **no reactive lymphocytes**

G. Other Conditions Associated with Lymphocytosis

1. **Viral**—hepatitis, influenza, mumps, measles, rubella, and varicella
2. **Nonviral**—*Bordetella pertussis* (whooping cough), brucellosis, toxoplasmosis

VI. MALIGNANT LEUKOCYTE DISORDERS

A. Basic Review

1. A malignant clone of cells proliferate that do not respond to normal regulatory mechanisms.
   a. **Leukemia** originates in the bone marrow and is initially **systemic.**
   b. **Lymphoma** originates in lymphoid tissue and is initially **localized.**
2. **Etiology remains unclear.** Multiple theories exist about **oncogene activation,** which most likely includes multiple factors:
   a. **Viral**—Viruses can suppress immune function or activate oncogenes (HTLV-I, II, V) and HIV-1.
   b. **Bone marrow damage**—Radiation, chemicals, and malignancies secondary to cancer treatments
   c. **Chromosome defects**—Some chromosomal abnormalities are diagnostic for leukemic subtypes; **t(15;17)** is diagnostic for **acute promyelocytic leukemia.**
   d. **Genetic factors**—Increased incidence in Down syndrome, Fanconi, and others
   e. **Immune dysfunction**—Hereditary and acquired defects in the immune system
3. Can be classified by **stem cell involved** and **length of clinical course**
   a. **Lymphoproliferative disorders**—acute or chronic
   b. **Myeloproliferative disorders**—acute or chronic
4. **Bone marrow examination** used to aid in diagnosis
   a. Indications include:
      1) Investigation of **peripheral blood abnormalities,** such as unexplained cytopenias
      2) **Staging** and **management** of patients with certain lymphomas or solid tumors
      3) **Ongoing monitoring** of response to therapy in patients with malignancy
   b. Optimal sample for examination includes both the **aspirate** and **core biopsy specimen**
   c. **Posterior superior iliac crest** most commonly used; less commonly used **anterior iliac crest** or **sternum**
   d. Routinely assessed for **cellularity, M:E ratio, megakaryocyte evaluation, iron stores, differential**
   e. Assessment may also include flow cytometry, cytogenetics, molecular, and microbiology testing

B. Comparison of Acute and Chronic Leukemias

1. **Duration**
   a. **Acute**—Survival is **weeks to months** without treatment; death is due to infection and bleeding.
   b. **Chronic**—Survival is **years** without treatment.
2. **Predominant cell type**
   a. **Acute**—Immature/blast cells predominate.
      1) **AML** has **myeloblasts.**
      2) **ALL** has **lymphoblasts.**
   b. **Chronic**—Maturing or mature cells predominate
      1) **CML** has **granulocytes.**
      2) **CLL** has **lymphocytes.**
3. **Clinical manifestations and laboratory findings**
   a. **Acute—sudden onset;** affects all ages
      1) Weakness and fatigue due to **anemia**
      2) Petechiae and bruising due to **thrombocytopenia**
      3) Fever and infection due to **neutropenia**
      4) Variable **leukocyte** count
      5) **Marrow blasts ≥20% based on World Health Organization classification or >30% based on French-American-British classification with cellularity >70%**
   b. **Chronic**—frequently **asymptomatic** initially; affects adults
      1) **Anemia** mild or absent
      2) Normal to slightly increased **platelet count**
      3) **WBC count** usually **high**
      4) Marrow cellularity is >70%.
   c. **Both acute and chronic**
      1) Unexplained weight loss or night sweats
      2) Splenomegaly, hepatomegaly, lymphadenopathy
4. **Treatment**
   a. **Chemotherapy** used is dependent on type of leukemia. Proper diagnosis is crucial.
   b. Radiation
   c. Bone marrow/stem cell transplant
   d. Supportive with transfusions of red blood cells and platelets, antibiotics, growth factors

C. French-American-British (FAB) and World Health Organization (WHO)

1. Hematopoietic malignancy classifications
2. **FAB classification** is based on **cellular morphology** and **cytochemical stain** results. FAB defines **acute leukemia as >30% bone marrow blasts.**
3. **WHO classification** is based on cellular morphology and cytochemical stains, but also utilizes information obtained from immunologic probes of **cell**

markers, cytogenetics, molecular abnormalities, and **clinical syndrome.** WHO defines **acute leukemia as ≥20% bone marrow blasts.**

4. **WHO classification** is now the **standard for diagnosis.**
5. **FAB classification** is easier to use and is still widely taught.

D. Cytochemical Stains—Used in Diagnosis of Hematologic Disorders

1. **Myeloperoxidase** (MPO)
   a. Cells of the **granulocytic series** and to a lesser degree the **monocytic series** contain the enzyme **peroxidase** in their granules that is detected by this stain. Auer rods also stain positive; **lymphocytic cells** are **negative** for this stain.
   b. Used to **differentiate blasts** of acute myelogenous leukemias **(AMLs)** from acute lymphoblastic leukemias **(ALLs)**
2. **Sudan black B**
   a. Stains **phospholipids** and **lipoproteins**
   b. **Granulocytic cells** and **Auer rods** stain **positive** (blue-black granulation); **lymphocytic cells** are **negative** for Sudan black B (reaction parallels MPO).
   c. Used to **differentiate blasts** of **AML from ALL**
3. **Esterases**
   a. **Specific esterase stain** (naphthol AS-D chloroacetate esterase stain)
      1) **Detects esterase** enzyme present in primary **granules** of **granulocytic cells; monocytic cells negative** for this stain
   b. **Nonspecific esterase stains** (alpha-naphthyl acetate and alpha-naphthyl butyrate)
      1) **Detects esterase** enzyme present in **monocytic cells; granulocytic cells negative** for these stains
   c. The **esterase stains** may be useful in **distinguishing acute leukemias** that are of **myeloid origin** (FAB M1, M2, M3, M4) **from** those **leukemias** that are primarily cells of **monocytic origin** (FAB M5).
4. **Periodic acid–Schiff (PAS)**
   a. PAS stains intracellular **glycogen** bright pink.
   b. Immature lymphoid cells, malignant erythroblasts, and megakaryocytic cells **stain positive** with this stain; myeloblasts and normal erythrocytic cells are **negative** with this stain.
   c. Useful in diagnosis of **erythroleukemia** (FAB M6) and **acute lymphoblastic leukemia**
5. **Leukocyte alkaline phosphatase (LAP)**
   a. Detects **alkaline phosphatase** enzyme activity in primary **granules** of **neutrophils**
   b. A positive stain will show dark precipitate when alkaline phosphatase activity is present; color is dependent on dye used.
   c. Used to differentiate **chronic myelogenous leukemia** (CML) from a **neutrophilic leukemoid reaction** (NLR)

d. **LAP score**
   1) 100 neutrophils are graded on a scale from 0 to 4+ based on stain intensity and size of granules. Results are added together.
   2) Reference range is 13–130.

e. **Clinical significance**
   1) **Decreased LAP score:** CML, paryoxysmal nocturnal hemoglobinuria
   2) **Normal LAP score:** CML in remission or with infection, Hodgkin lymphoma in remission, secondary polycythemia
   3) **Increased LAP score:** Neutrophilic leukemoid reaction, polycythemia vera, CML in blast crisis, late trimester pregnancy

6. **Tartrate-resistant acid phosphatase stain (TRAP)**
   a. Almost all blood cells contain the acid phosphatase enzyme and show positivity with acid phosphatase stain. Once tartrate is added, staining is inhibited in most cells.
   b. Only hairy cells from **hairy cell leukemia** are resistant to inhibition with tartrate and continue to stain positive; all other cells stain negative.

7. **Perl's Prussian blue stain**
   a. Free iron precipitates into small blue/green granules in mature erythrocytes; cells are called **siderocytes.** Iron inclusions are called siderotic granules or **Pappenheimer bodies** when visible with Wright's stain.
   b. **Sideroblasts** are nucleated RBCs in bone marrow that contain iron granules. These are normal. Ringed sideroblasts contain iron that encircles the nucleus. These are abnormal.
   c. **Increased percentage of siderocytes** is seen in severe hemolytic anemias (e.g., beta-thalassemia major), iron overload, sideroblastic anemia, and post-splenectomy; **ringed sideroblasts** are seen in bone marrow of myelodysplastic syndrome (refractory anemia with ringed sideroblasts [RARS]) and sideroblastic anemias.

E. Acute Lymphoproliferative Disorders

1. Unregulated proliferation of the **lymphoid stem cell;** classified morphologically using **FAB** criteria, or immunologically using **CD markers** to determine cell lineage (T or B cell)
2. **Clinical symptoms:** Fever, bone/joint pain, bleeding, hepatosplenomegaly
3. **Laboratory:** Neutropenia, anemia, and thrombocytopenia; variable WBC count, hypercellular marrow with bone marrow blasts $\geq 20\%$ (WHO) or $>30\%$ (FAB)
4. **Lymphoblasts** stain **PAS positive;** Sudan black B and myeloperoxidase negative
5. **FAB classification of acute lymphoblastic leukemia (ALL)**
   a. **FAB L1**
      1) Most common **childhood** leukemia (2- to 10-year peak); also found in young adults
      2) **Small lymphoblasts, homogeneous** appearance

3) Best prognosis
4) Most T cell ALLs are FAB L1.

b. **FAB L2**
1) Most common in **adults**
2) **Large lymphoblasts, heterogeneous** appearance

c. **FAB L3**
1) Leukemic phase of Burkitt lymphoma
2) Seen in both **adults and children**
3) **Lymphoblasts** are **large and uniform** with prominent nucleoli; cytoplasm stains deeply basophilic and may show vacuoles.
4) Poor prognosis
5) ALL FAB L3s are of **B cell lineage.**

d. **Burkitt lymphoma**
1) High-grade non-Hodgkin lymphoma phase of **FAB L3 leukemia**
2) Endemic in East Africa with high association with **Epstein-Barr virus;** children present with jaw/facial bone tumors
3) U.S. variant seen in children and young adults; present with abdominal mass

6. **Immunophenotyping of ALL**

a. **CD marker characteristics of B cell lineage**
1) Expressed by specific cell lines at different maturation stages; as cell matures, loses some antigens and expresses new ones
2) **Progenitor B cells** are **CD19, CD34,** and **TdT** (terminal deoxynucleotidyl transferase) **positive; CD10** (CALLA) **negative.** This is the least mature B cell.
3) **Early-pre–B cells ALL** are **CD10** (CALLA), **CD19, CD34,** and **TdT positive.** This is the most common subtype.
4) **Pre–B cells ALL** are **CD10** (CALLA), **CD19, CD20,** and **TdT positive.** This is the second most common subtype.
5) **B cells ALL** (early B) are **CD19, CD20 positive; TdT negative.** This is the most mature B cell and least common subtype.
6) **CD19** is the only marker **expressed** through **all stages of B cells.**

b. **CD marker characteristics of T cell lineage**
1) Differentiated from B cells using **markers present on all T cells, including CD2, CD3, CD5,** and **CD7** (pan T cell markers). **Immature T cells** are **TdT positive.**
2) **Immature T cells** can have **both or neither CD4 and CD8. Mature T cells** have **one or the other, but not both.**
3) **T cell ALL** occurs most often in **males; mediastinal mass** is a common finding.

7. **Genetic translocations** are helpful in diagnosis. Common ones include:

a. **FAB L3/Burkitt lymphoma—t(8;14)** with a rearrangement of the **MYC oncogene**

b. **Pre–B cell ALL** associated with **t(9;22); B cell ALL** associated with **t(4;11)**
c. **T cell ALL** associated with **t(7;11)**

F. Chronic Lymphoproliferative Disorders

1. **Chronic lymphocytic leukemia (CLL)**
   a. Found in adults over 60 years old; more common in males (2:1); survival rate of 5–10 years
   b. **B cell** malignancy **(CD19, CD20 positive)**
   c. Often asymptomatic and diagnosed secondary to other conditions
   d. **Laboratory:** Bone marrow **hypercellular;** blood shows absolute **lymphocytosis** of $>5.0 \times 10^9$/L; **homogeneous, small, hyperclumped lymphocytes** and **smudge cells**
   e. **Anemia** is **not usually present** unless **secondary to warm autoimmune hemolytic anemia** (frequent complication).
   f. **Small lymphocyte lymphoma** (SLL) is the lymphoma phase of CLL.
2. **Hairy cell leukemia (HCL)**
   a. Found in adults over 50 years old; more common in males (7:1)
   b. **B cell** malignancy **(CD19, CD20 positive)**
   c. Massive splenomegaly; extensive bone marrow involvement results in **dry tap** on bone marrow aspiration
   d. **Laboratory:** Pancytopenia; cytoplasm of lymphocytes shows hair-like projections; hairy cells are tartrate-resistant acid phosphatase (TRAP) stain positive
3. **Prolymphocytic leukemia (PLL)**
   a. Found in adults; more common in males
   b. Can be **either B cell** (most common) or **T cell** malignancy
   c. Marked splenomegaly
   d. **Laboratory:** Characterized by **lymphocytosis** ($>100 \times 10^9$/L) with many **prolymphocytes;** anemia and thrombocytopenia
   e. Both B and T cell types are aggressive and respond poorly to treatment.

G. Other Lymphoid Malignancies

1. **Plasma cell neoplasms**
   a. **Multiple myeloma**
      1) **Monoclonal gammopathy** causes **B cell** production of **excessive IgG** (most common) **or IgA,** with decreased production of the other immunoglobulins.
      2) Found in adults over 60 years old; incidence higher in males
      3) Multiple **skeletal system tumors** of **plasma cells** (myeloma cells) cause **lytic bone lesions** and hypercalcemia.
      4) Identified on serum protein electrophoresis by an "M"-spike in the gamma-globulin region; immunoglobulin class determined

using immunoelectrophoresis and quantified using an immunoassay method

5) Excessive IgG or IgA production by myeloma cells causes **increased blood viscosity.**

6) Abnormal immunoglobulin binds to platelets, blocking receptor sites for coagulation factor binding; this results in **prolonged bleeding**.

7) **Laboratory:** Bone marrow **plasma cells** >30%, marked rouleaux, increased erythrocyte sedimentation rate (ESR), blue background to blood smear, plasma cells and lymphocytes on blood smear

8) **Bence Jones** proteins **(free light chains—kappa or lambda)** found in the urine; toxic to tubular epithelial cells; cause **kidney damage**

b. **Waldenström macroglobulinemia**

1) **Monoclonal gammopathy** causes **B cell** production of **excessive IgM** (macroglobulin) and decreased production of the other immunoglobulins.

2) Found in adults over 60 years old

3) **Lymphadenopathy** and **hepatosplenomegaly;** no bone tumors

4) Identified on serum protein electrophoresis by an "M"-spike in the gamma-globulin region; immunoglobulin class determined using immunoelectrophoresis and quantified using an immunoassay method

5) Excessive IgM production causes **increased blood viscosity.**

6) Abnormal immunoglobulin may interfere with platelet function, fibrin polymerization, and the function of other coagulation proteins.

7) **Laboratory:** Marked rouleaux, increased ESR, blue background to blood smear; plasmacytoid lymphocytes, plasma cells, and lymphocytes on blood smear

2. **Lymphoma**

a. Proliferation of malignant cells in **solid lymphatic tissue**

b. Initially **localized; may spread** to bone marrow and blood

c. **Clinical symptom:** Lymphadenopathy

d. **Diagnosis:** Tissue biopsy, CD surface markers, cytogenetics, DNA analysis/PCR

e. World Health Organization **(WHO)** groups the lymphomas into **Hodgkin, B cell,** and **T/NK cell** (non-Hodgkin) neoplasms.

f. **Hodgkin lymphoma** (classical)

1) **40% of lymphomas;** seen in patients between 15 and 35 years of age and over 55 years of age; seen more frequently in males; certain subtypes have an **Epstein-Barr virus (EBV)** association

2) **Reed-Sternberg (RS)** cells found in lymph node biopsy are large, multinucleated cells each with prominent, large nucleoli; **B cell lineage**

3) Hodgkin lymphoma subtypes using **WHO** classification:

a) Nodular sclerosis—**70% are this subtype;** lowest EBV association

b) Mixed cellularity—20% are this subtype; **highest EBV association**

c) Lymphocyte rich
d) Lymphocyte depleted—uncommon
e) All subtypes are associated with RS cells

4) **Laboratory: Mild anemia, eosinophilia,** and **monocytosis; increased LAP score** and **ESR** during active disease

g. **Non-Hodgkin lymphoma**

1) **WHO** separates B cell and T/NK cell neoplasms into conditions with precursor cells or mature cells.
2) **60% of lymphomas;** seen in patients over 50 years of age; seen more frequently in males
3) Enlarged lymph nodes or gastrointestinal (GI) tumors
4) **B cell neoplasms** are more common; include Burkitt (lymphoma phase of Burkitt leukemia), mantle cell, follicular, and other lymphomas
5) Cells can be small and mature (e.g., small lymphocytic lymphoma) or large and primitive (e.g., Precursor B cell lymphoblastic lymphoma).
6) Can be slow growing or very aggressive

h. **Mycosis fungoides (cutaneous T cell lymphoma)**

1) Classified by WHO as a **T/NK cell neoplasm** (non-Hodgkin lymphoma)
2) Seen in patients over 50 years of age
3) **Cutaneous lymphoma** causes skin itching, leading to ulcerative tumors.
4) **Sézary syndrome,** a variant of mycosis fungoides, presents as a disseminated disease with widespread skin involvement and circulating lymphoma cells.
5) **CD2, CD3, and CD4 positive**

H. Acute Myeloproliferative Disorders

1. Unregulated proliferation of the **myeloid stem cell;** classified using morphology, cytochemical stains, CD markers, cytogenetics; **WHO classification standard for diagnosis; FAB** classification still widely taught
2. Platelets, erythrocytes, granulocytes, and/or monocytes can be affected.
3. Found mainly in **middle-aged adults;** also children <1 year old
4. **Clinical symptoms:** Fever, malaise, weight loss, petechiae, bruises, mild hepatosplenomegaly
5. **Laboratory: Neutropenia, anemia,** and **thrombocytopenia;** variable WBC count; **hypercellular marrow** with **bone marrow blasts ≥20% (WHO) or >30% (FAB)**
6. **Acute myelogenous leukemia** (AML)

a. **FAB M0—Blasts** exhibit myeloid markers **CD13, CD33,** and **CD34** but **stain negatively** with the usual cytochemical stains, myeloperoxidase (MPO), and Sudan black B (SBB). Constitutes <5% of AMLs.

b. **FAB M1 (AML without maturation)** shows **90%** or more **marrow myeloblasts;** may have **Auer rods** (fused primary granules)
c. **FAB M2 (AML with maturation)** shows **<90% marrow myeloblasts;** may have **Auer rods;** chromosome abnormality **t(8;21)**
   1) Both **FAB M1** and **FAB M2** are **SBB, MPO,** and **specific esterase positive.**
   2) FAB M1 and FAB M2 account for **50%** of the AMLs.
   3) **CD13** and **CD33 positive** (pan myeloid markers)
d. **Acute promyelocytic leukemia (APL; FAB M3)**
   1) Characterized by **>30% marrow promyelocytes** with bundles of **Auer rods (faggot cells);** heavy azurophilic granulation
   2) **Clinical symptoms:** Severe bleeding, hepatomegaly, and disseminated intravascular coagulation (promyelocytes have procoagulant activity)
   3) Accounts for 5% of the AMLs
   4) **SBB, MPO,** and **specific esterase positive**
   5) **CD13** and **CD33 positive; diagnostic** chromosome abnormality **t(15;17);** PML/RARA oncogene involved
e. **Acute myelomonocytic leukemia (AMML; FAB M4)**
   1) Characterized by **≥20% (WHO) or >30% (FAB) marrow myeloblasts** with **>20% cells of monocytic origin;** may have **Auer rods**
   2) Proliferation of unipotential stem cell **CFU-GM** that gives rise to both granulocytes and monocytes
   3) Accounts for 30% of the AMLs
   4) Increased urine/serum lysozyme
   5) **SBB, MPO, and specific** and **nonspecific esterase positive**
   6) **CD13** and **CD33 positive** (myeloid) and **CD14 positive** (monocytes)
   7) **M4Eo** is a **subclass of AMML** that presents with **eosinophilia.**
f. **Acute monocytic leukemia (AMoL; FAB M5)**
   1) Characterized by **≥20% (WHO) or >30% (FAB) marrow monoblasts**
   2) Accounts for 10% of the AMLs
   3) **Nonspecific esterase positive; CD14 positive**
   4) Contains two variants:
      a) **M5a** is seen in children with **>80% monoblasts** in the bone marrow.
      b) **M5b** is seen in middle-aged adults with **<80% monoblasts** in the bone marrow.
g. **Acute erythroleukemia (AEL, Di Guglielmo syndrome; FAB M6)**
   1) Characterized by **≥20% (WHO) or >30% (FAB) marrow myeloblasts** and **>50% dysplastic marrow normoblasts**
   2) Accounts for 5% of the AMLs
   3) **Malignant normoblasts** are **PAS positive.** The **myeloblasts** are **SBB and MPO positive.**

4) Malignant **normoblasts** are **CD45** and **CD71** (glycophorin A) **positive.** The **myeloblasts** are **CD13, CD15,** and **CD33 positive.**

h. **Acute megakaryocytic leukemia** (AMegL; FAB M7)

1) Characterized by a proliferation of **megakaryoblasts** and **atypical megakaryocytes** in the bone marrow; **blasts** may have **cytoplasmic blebs**
2) Accounts for <1% of the AMLs
3) Marrow aspiration results in dry tap; blood shows pancytopenia
4) Difficult to diagnose with cytochemical stains
5) **CD41, CD42,** and **CD61** (platelet markers) **positive**

i. **Bilineage leukemias** contain two cell populations. One population expresses **myeloid antigens;** the other population expresses **lymphoid antigens.**

j. **Biphenotypic leukemias** occur when **myeloid and lymphoid antigens** are expressed on the **same cell;** poor prognosis

k. The **WHO classification** of **acute myeloid leukemias** has more than **20 subtypes;** all have **≥20% marrow blasts.**

I. Chronic Myeloproliferative Disorders

1. Characterized by **hypercellular marrow, erythrocytosis, granulocytosis, and thrombocytosis**
   a. Defect of the **myeloid stem cell**
   b. Named for the cell line most greatly affected
   c. All may **terminate** in **acute leukemia.**
2. Molecular diagnostic studies are helpful in identifying oncogenes.
   a. **JAK2 oncogene** is implicated in polycythemia vera (80%), chronic idiopathic myelofibrosis (50%), and essential thrombocythemia (40%).
   b. The **BCR/ABL** oncogene is associated with chronic myelogenous leukemia.
3. **Chronic myelogenous leukemia** (CML) presents with **proliferation of granulocytes.**
   a. Found mainly in adults 45 years of age and older; often diagnosed secondary to other conditions
   b. **Clinical symptoms:** Weight loss, splenomegaly, fever, night sweats, and malaise
   c. Bone marrow has an **increased M:E ratio.**
   d. **Laboratory:** Blood findings include mild anemia and WBC between 50 and 500 $\times$ 10^9/L, with all stages of granulocyte production (shift to the left), including early forms of eosinophils and basophils. Myelocytes predominate; may have a few circulating blasts.
   e. CML can mimic a neutrophilic leukemoid reaction (NLR). LAP score is used to differentiate; **LAP** is **low in CML** and high in NLR.

f. **Philadelphia chromosome, t(9;22),** is present in virtually all patients. All cell lines are affected except lymphocytes. The few who lack the chromosome have a worse prognosis.
g. **Chronic phase** can last up to 5 years; **accelerated phase** (blast crisis) ultimately leads to acute leukemia in most patients. Recent therapies are improving the prognosis.

4. **Essential thrombocythemia (ET)**
   a. Characterized by **proliferation of megakaryocytes**
   b. Found mainly in adults 60 years of age and older
   c. **Laboratory:** Platelets commonly greater than 1000×10^9/L, giant forms, platelet function abnormalities, leukocytosis
   d. Must differentiate from reactive thrombocytosis and polycythemia vera
5. **Polycythemia vera (PV)**
   a. **Malignant hyperplasia** of the **multipotential myeloid stem cell** causes **increase in all cell lines** (polycythemia); **erythrocytes** most greatly **increased** despite **decreased erythropoietin** (EPO); inappropriate erythropoiesis
   b. **High blood viscosity** can cause high blood pressure, stroke, and heart attack.
   c. Found in adults 50 years of age and older
   d. **Laboratory:** Increased RBC ($7–10 \times 10^{12}$/L), hemoglobin (>20 g/dL), and hematocrit (>60%) along with increased leukocytes and platelets indicate polycythemia. RBC mass is increased with a normal plasma volume.
   e. **Treatment** is therapeutic phlebotomy, splenectomy, and chemotherapy. PV is a chronic disease with a life expectancy after diagnosis of up to 20 years.
   f. Must differentiate from other forms of polycythemia
      1) **Secondary polycythemia**
         a) **Increase in RBC mass** is an appropriate response to **increased EPO** or **tissue hypoxia.** Plasma volume, leukocyte count, and platelet count are normal.
         b) Can be caused by smoking, emphysema, or high altitude
      2) **Relative (pseudo-) polycythemia**
         a) **Decreased plasma volume** with a **normal RBC mass** caused by **dehydration** (diarrhea, diuretics, or burns)
         b) Increased **hemoglobin,** normal leukocyte and platelet count, **normal EPO**
6. **Chronic idiopathic myelofibrosis**
   a. **Myeloid stem cell disorder** characterized by **proliferation of erythroid, granulocytic,** and **megakaryocytic precursors** in marrow with dyspoiesis
   b. Progressive **marrow fibrosis**
   c. Found in adults 50 years of age and older
   d. **Clinical symptoms:** Bleeding due to abnormal platelet function; extramedullary hematopoiesis causes splenomegaly and hepatomegaly

e. **Laboratory:** Anisocytosis, poikilocytosis with teardrop cells, leukoerythroblastic anemia (immature neutrophils and nucleated RBCs in circulation); abnormal morphology associated with all cell lines

J. Myelodysplastic Syndromes (MDSs)

1. **Basic Review**
   a. Group of **acquired clonal disorders** affecting the **pluripotential stem cell;** characterized by progressive **blood cytopenias** despite **bone marrow hyperplasia**
   b. **Dyspoiesis** affects erythroid, myeloid, and megakaryocytic cell lines. High incidence of terminating in acute myelogenous leukemia occurs.
   c. MDS development can be triggered by chemotherapy, radiation, and chemicals.
   d. Found in older adults; rarely found in children and young adults
   e. Hematologic evidence of dyspoiesis:
      1) **Erythroid:** Variable anemia; erythrocytes can be macrocytic (with oval macrocytes) or microcytic and hypochromic; dimorphic erythrocytes, poikilocytosis, Howell-Jolly bodies, basophilic stippling, Cabot rings, nucleated RBCs
      2) **Myeloid:** Neutropenia, hypogranulation, hyposegmentation of neutrophils, shift to the left
      3) **Thrombocytes:** Variable platelet count, giant platelets, hypogranulation, micromegakaryocytes
   f. Five subgroups of MDS using the FAB classification scheme; **up to 30% blasts in the bone marrow**
2. **Refractory anemia (RA)**
   a. Anemia that is **refractory** (not responsive) **to therapy**
   b. **Laboratory:** Oval macrocytes, reticulocytopenia, dyserythropoiesis; bone marrow blasts <5% and peripheral blood blasts <1%
3. **Refractory anemia with ringed sideroblasts (RARS)**
   a. Ringed sideroblasts comprise more than 15% of bone marrow nucleated cells. Signs of dyserythropoiesis, neutropenia
   b. **Laboratory:** Similar to RA; dimorphic erythrocytes
   c. This is the primary/idiopathic sideroblastic anemia discussed with the anemias.
4. **Chronic myelomonocytic leukemia (CMML)**
   a. The one MDS that usually presents with **leukocytosis**
   b. **Laboratory:** Bone marrow blasts 5–20% and peripheral blood blasts <5%; absolute monocytosis greater than 1.0×10^9/L
5. **Refractory anemia with excess blasts (RAEB)**
   a. Trilineage cytopenias common
   b. **Laboratory:** Bone marrow and peripheral blood blasts are the **same as** with **CMML,** but there is **no** absolute monocytosis.
   c. The higher the blast percent, the worse the prognosis.

6. **Refractory anemia with excess blasts in transformation (RAEB-t)**
   a. **Laboratory:** bone marrow blasts >20% but less than 30%; peripheral blood blasts >5%
   b. **WHO** classification **reassigns RAEB-t as** an **acute leukemia** instead of a myelodysplastic syndrome because of the bone marrow blast percent.
7. WHO classification of MDS has additional groups (e.g., refractory cytopenia with multilineage dysplasia, 5q deletion syndrome).
8. WHO created the new category of **myelodysplastic/myeloproliferative disease,** which includes the FAB's **CMML.**

VII. ERYTHROCYTES

A. General Characteristics

1. **Oxygen transport, removal of metabolic waste**
2. **Loss of nucleus** is required for function.
3. Normal life span is **120 days.**

B. Erythropoietin

1. Produced mainly by the **kidneys**
2. **Growth factor** that **stimulates erythrocyte production from myeloid progenitor cell;** influences colony-forming unit-erythrocytes (CFU-Es) to differentiate into erythroblasts

C. Erythrocyte Maturation

1. **Pronormoblast (rubriblast)**
   a. Earliest RBC, size up to 20 μm, with an N:C ratio of 8:1
   b. 1–3 nucleoli, nucleus has dark areas of DNA
   c. Chromatin is fine and uniform, and stains intensely
   d. Deep blue cytoplasm with no granules
2. **Basophilic normoblast (prorubricyte)**
   a. Size up to 16 μm with an N:C ratio of 6:1
   b. Centrally located nucleus with 0–1 nucleoli
   c. Chromatin is coarsening.
   d. Cytoplasm is less blue but intensely basophilic (RNA).
3. **Polychromatophilic normoblast (rubricyte)**
   a. Size up to 12 μm with an N:C ratio of 4:1
   b. Eccentric nucleus with no nucleoli
   c. Chromatin shows significant clumping.
   d. Begins to produce hemoglobin, resulting in gray-blue cytoplasm
4. **Orthochromic normoblast (metarubricyte)**
   a. Size up to 10 μm with an N:C ratio of 0.5:1
   b. Eccentric nucleus with small, fully condensed (pyknotic) nucleus; no nucleoli
   c. Pale blue to salmon cytoplasm
   d. Hemoglobin synthesis decreases

5. **Reticulocyte**
   a. Size up to 10 μm
   b. A reticulocyte contains **no nucleus** but has mitochondria and ribosomes.
   c. Last stage to synthesize hemoglobin
   d. Last stage in bone marrow before release to the blood
   e. Reference ranges are 0.5–1.5% for adults and 2.5–6.5% for newborns, with slightly increased ranges at higher altitudes.
   f. A **supravital stain** is used to enumerate reticulocytes.
   g. **Reticulocyte count** is one of the best indicators of bone marrow function.
   h. **Stress reticulocytes** are young cells released from bone marrow after older reticulocytes have been released. This is a response to increased need.
   i. Hemoglobin continues to be produced by reticulocytes for approximately 24 hours after exiting the bone marrow.
6. **Mature erythrocyte**
   a. Size range is 6–8 μm.
   b. Round, biconcave discocyte
   c. Salmon with central pallor (clearing in the center) when a blood smear is Wright's stained
      1) **Normal cells** have a **central pallor** that is one-third the diameter of the cell.
      2) **Decreased central pallor** is seen with **spherocytic** disorders, including thermal injury and liver disease.
      3) **Central pallor greater** than one-third the diameter of the cell is seen in **microcytic anemias.**
   d. **RBC reference ranges** in SI units:
      1) Females $4.0–5.4 \times 10^{12}$/L (conventional units $4.0–5.4 \times 10^6/\mu L$)
      2) Males $4.6–6.0 \times 10^{12}$/L (conventional units $4.6–6.0 \times 10^6/\mu L$)
   e. Erythropoiesis is regulated by **erythropoietin** produced in the kidney. Additional regulation includes:
      1) Hypoxia due to high altitudes, heart or lung dysfunction, anemia
      2) Androgens (male hormones that appear to enhance the activity of erythropoietin) and hemolytic anemias (increased erythrocyte destruction)

D. Erythrocyte Physiology

1. Early RBCs get energy from oxidative phosphorylation. During maturation, the mitochondria are lost, and energy is derived from glycolysis.
2. Erythrocytes need proper volume ratio for exchange of blood gases and flexibility to travel through capillaries. This is accomplished by the cation pump, a mechanism that keeps sodium outside and potassium inside the cell.
3. Erythrocyte membrane is 50–60% lipid (phospholipids, cholesterol, and glycolipids) and 40–50% protein.

E. Substances Needed for Erythropoiesis

1. **Iron:** Must be in the ferrous state (Fe^{2+}) to transport oxygen
2. **Amino acids:** Globin-chain synthesis
3. **Folic acid/vitamin B_{12}:** DNA replication/cell division
4. **Others:** Erythropoietin, vitamin B_6 (pyridoxine), trace minerals

F. Erythrocytic Morphology and Associated Disease (Size and Shape)

1. **Normocytes (discocytes)** are normal erythrocytes that are approximately the same size as the nucleus of a small lymphocyte.
2. **Macrocytes**
   a. RBCs greater than 8 μm in diameter; **MCV greater than 100 fL**
   b. Seen in **megaloblastic anemias,** such as B_{12}/folate deficiency
   c. Seen in non-megaloblastic anemia of liver disease or accelerated erythropoiesis; also seen in normal newborns
3. **Microcytes**
   a. RBCs less than 6 μm in diameter; **MCV less than 80 fL**
   b. Seen in iron-deficiency anemia, thalassemias, sideroblastic anemia, and anemia of chronic disease
4. **Anisocytosis**
   a. **Variation in RBC size,** indicating a heterogeneous RBC population (dimorphism)
   b. Correlates with RDW (red blood cell distribution width), especially when the RDW exceeds 15.0%
   c. Seen post-transfusion, post-treatment for a deficiency (e.g., iron), presence of two concurrent deficiencies (e.g., iron and vitamin B_{12}), and idiopathic sideroblastic anemia
5. **Poikilocytosis**
   a. General term to describe **variation in shape**
   b. Associated with a variety of pathologic conditions
6. **Echinocytes** include **crenated** and **burr cells**
   a. Have evenly spaced **round** projections; central pallor area present
   b. Seen in liver disease, uremia, heparin therapy, pyruvate kinase deficiency, or as artifact
   c. Caused by changes in osmotic pressure
7. **Acanthocytes (spur cells)**
   a. Have unevenly spaced **pointed** projections; **lack** a central pallor area
   b. Associated with alcoholic liver disease, post-splenectomy, and abetalipoproteinemia
   c. Caused by excessive cholesterol in the membrane
8. **Target cells (codocytes or Mexican hat cells)**
   a. Show a central area of hemoglobin surrounded by a colorless ring and a peripheral ring of hemoglobin; cells have an **increased surface-to-volume ratio**

b. Seen in liver disease, hemoglobinopathies, thalassemia, iron-deficiency anemia
c. Caused by excessive cholesterol in the membrane or a hemoglobin distribution imbalance

9. **Spherocytes**
   a. Disk-shaped cell with a smaller volume than a normal erythrocyte; cells have a **decreased surface-to-volume ratio**
   b. **Lack** a central pallor area
   c. Associated with defects of the red cell membrane proteins
   d. **MCHC may be >37%;** increased osmotic fragility
   e. **Damaged RBC;** seen in hereditary spherocytosis, G6PD deficiency, and immune hemolytic anemias
   f. **Microspherocytes** (<4 μm) are frequently seen in severe thermal injury (burns).
10. **Teardrops (dacryocytes)**
    a. **Pear-shaped** cell with **one blunt projection**
    b. Seen in megaloblastic anemias, thalassemia, and extramedullary hematopoiesis (myelofibrosis, myelophthisic anemia)
11. **Sickle cells (drepanocytes)**
    a. Shapes vary but show **thin, elongated, pointed ends** and will appear **crescent shaped;** usually lack a central pallor area
    b. Contain polymers of abnormal **hemoglobin S**
    c. Seen in hemoglobinopathies SS, SC, SD, and S/β-thalassemia
    d. Cell shape is caused by **cell membrane alterations** due to an amino acid substitution
12. **Helmet cells (horn cells or keratocytes)**
    a. Interior portion of cell is hollow, resembling a horn or helmet
    b. Seen in microangiopathic hemolytic anemias
13. **Schistocytes (RBC fragments)**
    a. **Damaged RBC;** fragments of various sizes and shapes are present, often with **pointed projections**
    b. Seen in microangiopathic hemolytic anemias (e.g., DIC, HUS, TTP), thermal injury, renal transplant rejection, and G6PD deficiency
14. **Stomatocytes (mouth cells)**
    a. Characterized by an elongated or slit-like area of central pallor
    b. Seen in liver disease, hereditary stomatocytosis, or as artifact
    c. Caused by osmotic changes due to cation imbalance (Na^+/K^+)
15. **Elliptocytes (ovalocytes)**
    a. Cigar- to egg-shaped erythrocytes
    b. Associated with defects of the red cell membrane proteins
    c. Seen in hereditary elliptocytosis, iron-deficiency anemia (pencil forms), megaloblastic anemia (macro-ovalocytes), thalassemia major

G. Erythrocyte Inclusions and Associated Diseases

1. **Nucleated RBCs (nRBCs, nucRBCs)**
   a. Usually **orthochromic normoblasts** (metarubricyte) but can appear in any erythrocytic stage of maturation
   b. Indicate **bone marrow stimulation** or **increased erythropoiesis**
   c. Associated with thalassemia major, sickle cell anemia, and other hemolytic anemias, erythroleukemia, and myeloproliferative disorders
   d. Normal newborns can have a few.
   e. Healthy individuals should have **none** on a peripheral blood smear.
2. **Howell-Jolly bodies**
   a. Small, round **DNA fragments** (0.5–1.0 μm in diameter) usually **one** per cell, but can be **multiple**
   b. Stain **dark purple to black** with **Wright's stain**
   c. Not seen in normal erythrocytes; normally **pitted** by splenic macrophages
   d. Seen in sickle cell anemia, beta-thalassemia major, and other severe hemolytic anemias, megaloblastic anemia, alcoholism, post-splenectomy
3. **Basophilic stippling**
   a. Multiple, tiny, fine, or coarse **inclusions** (ribosomal RNA remnants) **evenly dispersed throughout the cell;** "blueberry bagel" appearance
   b. Stain **dark blue** with **Wright's stain**
   c. Seen in thalassemias, megaloblastic anemias, sideroblastic anemia, lead poisoning, and alcoholism
4. **Pappenheimer bodies**
   a. Small, irregular, **dark-staining iron granules** usually **clumped together at periphery of the cell**
   b. Stain with **Perl's Prussian blue stain;** appear **dark violet with Wright's stain**
   c. Caused by an accumulation of ribosomes, mitochondria, and iron fragments
   d. Seen in sideroblastic anemia, hemoglobinopathies, thalassemia, megaloblastic anemia, myelodysplastic syndrome (RARS)
5. **Cabot rings**
   a. Thin, **red-violet,** single to multiple **ringlike structures** that may appear in **loop or figure-eight shapes**
   b. Seen in megaloblastic anemia, myelodysplastic syndromes, lead poisoning
   c. Composed of **fragments of nuclear material**
6. **Hemoglobin C crystals**
   a. Condensed, intracellular, rod-shaped crystal
   b. Seen in hemoglobin C or SC disease, but not in trait
7. **Hemoglobin SC crystals (Washington monument)**
   a. 1–2 blunt, fingerlike projections extending from the cell membrane
   b. Seen in hemoglobin SC disease

8. **Heinz bodies**
   a. Multiple inclusions ranging in size from 0.3 to 2.0 μm
   b. Invisible with Wright's stain; must use a **supravital stain to visualize**
   c. Seen in G6PD deficiency, beta-thalassemia major, Hgb H disease, unstable hemoglobinopathies, drug-induced anemias
   d. Represent **denatured hemoglobin**
9. **Malarial parasites**—include *P. vivax, P. falciparum, P. malariae* and *P. ovale*

H. Erythrocyte Hemoglobin Content and Associated Diseases

1. **Normochromasia:** Cells have the normal one-third clear, central pallor area
2. **Hypochromasia**
   a. Central pallor area is greater than one-third the diameter of the cell
   b. **MCH** and **MCHC** usually **decreased**
   c. Often associated with **microcytosis**
   d. Seen in iron-deficiency anemia, thalassemias, anemia of chronic disease, sideroblastic anemia, myelodysplastic syndromes
3. **Polychromasia**
   a. Variation in hemoglobin content showing a **slight blue tinge** when stained with **Wright's stain; residual RNA**
   b. Indicates **reticulocytosis;** supravital reticulocyte stain to enumerate
   c. Usually slightly macrocytic
4. **Hyperchromasia** (term no longer used)
   a. Current terminology is **spherocyte;** lacks a central pallor area

I. Abnormal Erythrocyte Distributions and Associated Diseases

1. **Rouleaux**
   a. **Stacking** or "coining" pattern of erythrocytes due to **abnormal or increased plasma proteins**
   b. May see excessively blue color to smear macroscopically and microscopically
   c. Seen in hyperproteinemia, multiple myeloma, Waldenström macroglobulinemia, and conditions that produce increased fibrinogen (chronic inflammation)
   d. May be artifact; considered normal in thicker area of the peripheral smear
   e. True rouleaux formation is determined in the thin area of the peripheral smear.
2. **Agglutination**
   a. Characterized by clumping of erythrocytes with **no pattern**
   b. Occurs when **erythrocytes** are **coated with IgM antibodies and complement**
   c. Seen in cold autoimmune hemolytic anemia (cold agglutinin disease)
   d. Warm blood to 37°C to correct a false low RBC and hematocrit, and false high MCHC (>37 g/dL) when using an automated cell counting instrument.

VIII. HEMOGLOBIN

A. Introduction

1. Hemoglobin is an **oxygen-transporting protein** contained within erythrocytes.
2. The heme portion of hemoglobin gives erythrocytes their characteristic red color.

B. Hemoglobin Structure

1. **Four identical heme** groups, each consisting of a **protoporphyrin ring** and **ferrous (Fe^{2+}) iron**
2. **Four globin** (polypeptide) **chains**
   a. Alpha chains have 141 amino acids.
   b. Beta, gamma, and delta chains have 146 amino acids.
3. The **amino acid sequence** of the globin chain determines the type of hemoglobin; normal adult hemoglobin consists of two alpha and two non-alpha chains in pairs.

C. Hemoglobin Synthesis

1. 65% hemoglobin synthesis occurs in immature nRBCs.
2. 35% hemoglobin synthesis occurs in reticulocytes.
3. **Heme synthesis** occurs in the **mitochondria of normoblasts** and is dependent on glycine, succinyl coenzyme A, aminolevulinic acid synthetase, and vitamin B_6 (pyridoxine).
4. **Globin synthesis** occurs in the **ribosomes,** and it is controlled on **chromosome 16** for alpha chains and **chromosome 11** for all other chains.
5. Each **globin** chain **binds to a heme** molecule in the cytoplasm of the immature RBC.

D. Hemoglobin/Erythrocyte Breakdown

1. **Intravascular hemolysis** (10%)
   a. Occurs when **hemoglobin breaks down in the blood** and **free hemoglobin** is released into **plasma**
   b. **Free hemoglobin** binds to **haptoglobin** (major free hemoglobin transport protein), **hemopexin,** and **albumin,** and it is phagocytized by liver macrophages.
   c. **Laboratory:** Increased plasma hemoglobin, serum bilirubin, serum LD, and urine urobilinogen; hemoglobinuria and hemosiderinuria present; decreased serum haptoglobin
2. **Extravascular hemolysis** (90%)
   a. Occurs when **senescent/old RBCs** are **phagocytized** by macrophages in the liver or spleen
   b. **Protoporphyrin ring** metabolized to **bilirubin** and **urobilinogen;** excreted in urine and feces
   c. **Globin chains** are recycled into the **amino acid pool** for protein synthesis.

d. **Iron** binds to **transferrin** and is transported to bone marrow for new RBC production, or it is stored for future use in the form of **ferritin** or **hemosiderin.**

E. Hemoglobin and Iron

1. Most iron in the body is in hemoglobin and must be in the ferrous state (Fe^{2+}) to be used. **Fe^{2+} binds to oxygen** for transport to lungs and body tissues. Ferric iron (Fe^{3+}) is not able to bind to hemoglobin, but does bind to transferrin. Iron is an **essential** mineral and is not produced by the body.
   a. **Serum iron** measures the amount of Fe^{3+} bound to transferrin.
   b. **Total iron-binding capacity (TIBC)** measures the total amount of iron that transferrin can bind when fully saturated.
   c. Serum ferritin is an indirect measurement of storage iron in tissues and bone marrow.

F. Types of Hemoglobin

1. **Hgb F** contains two alpha- and two gamma-globin chains. Hgb F functions in a reduced oxygen environment. **Hgb F predominates at birth** (80%). Gamma chain production switches over to beta chain production and is complete by 6 months of age.
   a. **Laboratory:** Alkali denaturation test and Kleihauer-Betke acid elution stain (Hgb F is resistant to denaturation/elution), column chromatography, radial immunodiffusion
   b. Hgb F is a compensatory hemoglobin and can be increased in homozygous hemoglobinopathies and beta-thalassemia major.
2. **Adult**
   a. **Hgb A** contains two alpha- and two beta-globin chains.
      1) Hgb A is subdivided into glycosylated fractions. **A_{1c}** fraction reflects glucose levels in the blood and is used to monitor individuals with diabetes mellitus.
   b. **Hgb A_2** contains two alpha- and two delta-globin chains.
   c. Reference range for a normal adult is **97% Hb A, 2% Hb A_2, and 1% Hb F.**

G. Different Forms of Normal Hemoglobin

1. **Oxyhemoglobin:** Hemoglobin with $Fe^{2+} + O_2$; seen in arterial circulation
2. **Deoxyhemoglobin:** Hemoglobin with Fe^{2+} but no O_2; seen in venous circulation
3. **Carboxyhemoglobin:** Hemoglobin with Fe^{2+} and carbon monoxide (CO); hemoglobin has 200× more affinity for CO than O_2, so CO is carried instead of O_2; can result in death, but is reversible if given pure O_2
4. **Sulfhemoglobin:** Hemoglobin with S; cannot transport O_2; seldom reaches fatal levels; caused by drugs and chemicals; irreversible, not measured by the cyanmethemoglobin method
5. **Methemoglobin:** Hemoglobin with Fe^{3+}; cannot transport O_2; increased levels cause cyanosis and anemia

H. Oxygen Dissociation Curve

1. **Oxygen affinity** is the ability of hemoglobin to bind or release oxygen. Expressed in terms of the oxygen tension at which hemoglobin is 50% saturated with oxygen.
2. The relationship between oxygen tension and hemoglobin saturation with oxygen is described by the **oxygen dissociation curve.**
   a. **Right shift** decreases oxygen affinity, **more** O_2 release to the tissues—high 2,3-bisphosphoglycerate (formerly 2,3-diphosphoglycerate/2,3-DPG) level or increased body temperature; decreased body pH
   b. **Left shift** increases oxygen affinity, **less** O_2 release to the tissues—low 2,3-bisphosphoglycerate (2,3-BPG) level or decreased body temperature; increased body pH

IX. ANEMIAS

A. Introduction: Anemia is defined as a decrease in erythrocytes and hemoglobin, resulting in decreased oxygen delivery to the tissues. The anemias can be classified **morphologically** using RBC indices (MCV, MCH, and MCHC). They can also be classified based on **etiology/cause.** Anemia is suspected when the hemoglobin is **<12 g/dL in men** or **<11 g/dL in women.**

1. **Relative (pseudo) anemia**
   a. RBC mass is normal, but **plasma volume** is **increased.**
   b. Secondary to an unrelated condition and can be transient in nature
   c. Causes include conditions that result in hemodilution, such as pregnancy and volume overload.
   d. **Reticulocyte** count **normal; normocytic/normochromic** anemia
2. **Absolute anemia**
   a. **RBC mass** is **decreased,** but plasma volume is normal. This is indicative of a true decrease in erythrocytes and hemoglobin.
   b. **Mechanisms** involved include:
      1) **Decreased delivery** of red cells into circulation
         a) Caused by impaired or defective production
         b) Bone marrow fails to respond; **reticulocytopenia**
      2) **Increased loss** of red cells from the circulation
         a) Caused by acute bleeding or accelerated destruction (hemolytic)
         b) Bone marrow can respond; **reticulocytosis**

B. Impaired or Defective Production Anemias

1. **Iron-deficiency anemia**
   a. Most common form of anemia in the United States
   b. Prevalent in infants and children, pregnancy, excessive menstrual flow, elderly with poor diets, malabsorption syndromes, chronic blood loss (GI blood loss, hookworm infection)

   c. **Laboratory: Microcytic/hypochromic anemia;** serum iron, ferritin, hemoglobin/hematocrit, RBC indices, and reticulocyte count low; RDW and total iron-binding capacity (TIBC) high; smear shows ovalocytes/pencil forms
   d. **Clinical symptoms:** Fatigue, dizziness, pica, stomatitis (cracks in the corners of the mouth), glossitis (sore tongue), and koilonychia (spooning of the nails)
2. **Anemia of chronic disease (ACD)**
   a. Due to an **inability to use available iron** for hemoglobin production
   b. Impaired release of storage iron associated with increased **hepcidin** levels
      1) Hepcidin is a liver hormone and a positive acute-phase reactant. It plays a major role in body iron regulation by influencing intestinal iron absorption and release of storage iron from macrophages.
      2) Inflammation and infection cause hepcidin levels to increase; this decreases release of iron from stores.
   c. **Laboratory: Normocytic/normochromic anemia,** or **slightly microcytic/hypochromic anemia;** increased ESR; normal to increased ferritin; low serum iron and TIBC
   d. Associated with persistent infections, chronic inflammatory disorders (SLE, rheumatoid arthritis, Hodgkin lymphoma, cancer)
   e. Anemia of chronic disease is second only to iron deficiency as a common cause of anemia
3. **Sideroblastic anemia**
   a. Caused by **blocks** in the **protoporphyrin pathway** resulting in defective hemoglobin synthesis and iron overload
   b. Excess iron accumulates in the mitochondrial region of the immature erythrocyte in the bone marrow and encircles the nucleus; cells are called **ringed sideroblasts.**
   c. Excess iron accumulates in the mitochondrial region of the mature erythrocyte in circulation; cells are called **siderocytes;** inclusions are siderotic granules (**Pappenheimer bodies** on Wright's stained smears).
   d. **Siderocytes** are best demonstrated using **Perl's Prussian blue stain.**
   e. Two types of sideroblastic anemia:
      1) **Primary**—irreversible; cause of the blocks unknown
         a) Two RBC populations **(dimorphic)** are seen.
         b) This is one of the myelodysplastic syndromes—refractory anemia with ringed sideroblasts (RARS).
      2) **Secondary**—reversible; causes include alcohol, anti-tuberculosis drugs, chloramphenicol
   f. **Laboratory: Microcytic/hypochromic anemia** with increased ferritin and serum iron; TIBC is decreased
4. **Lead poisoning**
   a. **Multiple blocks** in the **protoporphyrin pathway** affect heme synthesis.
   b. Seen mostly in children exposed to lead-based paint

c. **Clinical symptoms:** Abdominal pain, muscle weakness, and a **gum lead line** that forms from blue/black deposits of lead sulfate
d. **Laboratory: Normocytic/normochromic anemia** with characteristic coarse **basophilic stippling**

5. **Porphyrias**
   a. These are a group of **inherited disorders** characterized by a **block** in the **protoporphyrin pathway** of heme synthesis. Heme precursors before the block accumulate in the tissues, and large amounts are excreted in urine and/or feces.
   b. **Clinical symptoms:** Photosensitivity, abdominal pain, CNS disorders
   c. Hematologic findings are insignificant.
6. **Megaloblastic anemias**
   a. **Defective DNA synthesis** causes abnormal nuclear maturation; RNA synthesis is normal, so the cytoplasm is not affected. The nucleus matures slower than the cytoplasm (asynchronism). **Megaloblastic** maturation is seen.
   b. Caused by either a vitamin **B_{12}** or **folic acid deficiency**
   c. **Laboratory:** Pancytopenia, **macrocytic/normochromic anemia** with oval macrocytes and teardrops, hypersegmented neutrophils; inclusions include Howell-Jolly bodies, nucleated RBCs, basophilic stippling, Pappenheimer bodies, and Cabot rings; elevated LD, bilirubin, and iron levels due to destruction of fragile, megaloblastic cells in the blood and bone marrow
   d. **Vitamin B_{12} deficiency (cobalamin)**
      1) Intrinsic factor is secreted by parietal cells and is needed to bind vitamin B_{12} for absorption into the intestine.
         a) **Pernicious anemia:** Caused by deficiency of **intrinsic factor,** antibodies to intrinsic factor, or antibodies to parietal cells
         b) Prevalent in older adults of English, Irish, and Scandinavian descent
         c) Characterized by **achlorhydria** and atrophy of gastric parietal cells
      2) Other causes of vitamin B_{12} deficiency include malabsorption syndromes, *Diphyllobothrium latum* tapeworm, total gastrectomy, intestinal blind loops, and a total vegetarian diet.
      3) **Clinical symptoms:** Jaundice, weakness, sore tongue (glossitis), and gastrointestinal (GI) disorder, numbness and other **CNS problems**
      4) Vitamin B_{12} deficiency takes 3–6 years to develop because of high body stores.
   e. **Folic acid deficiency** causes a **megaloblastic anemia** with a blood picture and clinical symptoms similar to vitamin B_{12} deficiency, except there is **no CNS involvement.** It is associated with poor diet, pregnancy, or chemotherapeutic anti-folic acid drugs such as **methotrexate.** Folic acid has low body stores.
7. **Non-megaloblastic macrocytic anemias** include alcoholism, liver disease, and conditions that cause accelerated erythropoiesis. The **erythrocytes** are **round,** not oval as is seen in the megaloblastic anemias.

8. **Aplastic anemia**
   a. Bone marrow failure causes **pancytopenia.**
   b. **Laboratory:** Decrease in hemoglobin/hematocrit and reticulocytes; **normocytic/normochromic anemia;** no response to erythropoietin
   c. Most commonly affects people around the age of 50 and above. It can occur in children.
   d. Patients have a poor prognosis with complications that include bleeding, infection, and iron overload due to frequent transfusion needs.
   e. **Treatment** includes bone marrow or stem cell transplant and immunosuppression.
   f. Can be genetic, acquired, or idiopathic
      1) **Genetic aplastic anemia (Fanconi anemia)**
         a) **Autosomal recessive** trait
         b) Dwarfism, renal disease, mental retardation
         c) Strong association with malignancy development, especially acute lymphoblastic leukemia
      2) **Acquired aplastic anemia** (secondary) caused by:
         a) Antibiotics: Chloramphenicol and sulfonamides
         b) Chemicals: Benzene and herbicides
         c) About 30% of acquired aplastic anemias are due to **drug exposure.**
         d) Viruses: B19 parvovirus secondary to hepatitis, measles, CMV, and Epstein-Barr virus
         e) Radiation or chemotherapy
         f) Myelodysplastic syndromes, leukemia, solid tumors, paroxysmal nocturnal hemoglobinuria
      3) **Idiopathic** (primary): 50–70% of aplastic anemias have **no known cause.**
   g. Diamond-Blackfan anemia
      1) **True red cell aplasia** (leukocytes and platelets normal in number)
      2) Autosomal inheritance
9. **Myelophthisic** (marrow replacement) **anemia**
   a. **Hypoproliferative anemia** caused by replacement of bone marrow hematopoietic cells by malignant cells or fibrotic tissue
   b. Associated with cancers (breast, prostate, lung, melanoma) with bone metastasis
   c. **Laboratory: Normocytic/normochromic anemia;** leukoerythroblastic blood picture

C. Blood Loss Anemia

1. **Acute blood loss anemia**
   a. Characterized by a **sudden loss of blood** resulting from trauma or other severe forms of injury
   b. **Clinical symptoms:** Hypovolemia, rapid pulse, low blood pressure, pallor

c. **Laboratory: Normocytic/normochromic anemia;** initially normal reticulocyte count, hemoglobin/hematocrit; in a few hours, increase in platelet count and leukocytosis with a left shift, drop in hemoglobin/hematocrit and RBC; reticulocytosis in 3–5 days

2. **Chronic blood loss anemia**
   a. Characterized by a **gradual, long-term loss of blood;** often caused by gastrointestinal bleeding
   b. **Laboratory: Initially normocytic/normochromic anemia** that over time causes a decrease in hemoglobin/hematocrit; **gradual loss** of **iron** causes **microcytic/hypochromic anemia**

D. Hemolytic Anemias Due to Intrinsic Defects

1. All cause a **normocytic/normochromic anemia;** usually hereditary with reticulocytosis due to accelerated destruction
2. **Hereditary spherocytosis**
   a. **Most common membrane defect;** autosomal dominant; characterized by splenomegaly, variable degree of anemia, **spherocytes** on the peripheral blood smear
   b. Increased permeability of the membrane to sodium
   c. Results in loss of membrane fragments; erythrocytes have **decreased surface area-to-volume ratio;** rigid spherocytes culled/removed by splenic macrophages
   d. **Laboratory:** Spherocytes, **MCHC may be >37 g/dL,** increased osmotic fragility, and increased serum bilirubin
3. **Hereditary elliptocytosis (ovalocytosis)**
   a. Autosomal dominant; most persons **asymptomatic** due to normal erythrocyte life span; **>25% ovalocytes** on the peripheral blood smear
   b. Membrane defect is caused by polarization of cholesterol at the ends of the cell rather than around pallor area.
4. **Hereditary stomatocytosis**
   a. Autosomal dominant; variable degree of anemia; **up to 50% stomatocytes** on the blood smear
   b. Membrane defect due to abnormal permeability to both sodium and potassium; causes erythrocyte swelling
5. **Hereditary acanthocytosis (abetalipoproteinemia)**
   a. Autosomal recessive; mild anemia associated with steatorrhea, neurological and retinal abnormalities; 50–100% of erythrocytes are **acanthocytes**
   b. Increased cholesterol:lecithin ratio in the membrane due to abnormal plasma lipid concentrations; absence of serum β-lipoprotein needed for lipid transport
6. **G6PD (glucose-6-phosphate dehydrogenase) deficiency**
   a. **Sex-linked enzyme defect;** most common enzyme deficiency in the **hexose monophosphate shunt**

b. Reduced glutathione levels are not maintained because of decreased NADPH generation.
c. Results in oxidation of hemoglobin to **methemoglobin** (Fe^{3+}); denatures to form **Heinz bodies**
d. Usually, not anemic until oxidatively challenged (primaquine, sulfa drugs); then severe hemolytic anemia with reticulocytosis

7. **Pyruvate kinase (PK) deficiency**
   a. Autosomal recessive; most common enzyme deficiency in **Embden-Meyerhof pathway**
   b. Lack of ATP causes impairment of the cation pump that controls intracellular sodium and potassium levels.
   c. Decreased erythrocyte deformability reduces their life span.
   d. Severe hemolytic anemia with reticulocytosis and echinocytes
8. **Paroxysmal nocturnal hemoglobinuria (PNH)**
   a. An **acquired** membrane defect in which the red cell membrane has an increased sensitivity for **complement binding** as compared to normal erythrocytes
   b. Etiology unknown
   c. All cells are abnormally sensitive to lysis by complement.
   d. **Characterized by:** Pancytopenia; chronic intravascular hemolysis causes hemoglobinuria and hemosiderinuria at an acid pH at night; PNH noted for low leukocyte alkaline phosphatase (LAP) score; Ham's and sugar water tests used in diagnosis; increased incidence of acute leukemia
   e. Although Ham's and sugar water tests have been traditionally used in diagnosis of PNH, the standard now used is flow cytometry to detect deficiencies for surface expression of glycosyl phosphatidylinositol (GPI)-linked proteins such as CD55 and CD59.

E. Hemolytic Anemias Due to Extrinsic/Immune Defects

1. All cause a **normocytic/normochromic anemia** due to defects extrinsic to the RBC. All are acquired disorders that cause **accelerated destruction** with **reticulocytosis.**
2. **Warm autoimmune hemolytic anemia (WAIHA)**
   a. RBCs are coated with **IgG and/or complement.** Macrophages may phagocytize these RBCs, or they may remove the antibody or complement from the RBC's surface, causing membrane loss and spherocytes.
   b. 60% of cases are idiopathic; other cases are secondary to diseases that alter the immune response (e.g., chronic lymphocytic leukemia, lymphoma); can also be drug induced.
   c. **Laboratory:** Spherocytes, MCHC may be >37 g/dL, increased osmotic fragility, bilirubin, reticulocyte count; occasional nRBCs present; positive direct antiglobulin test (DAT) helpful in differentiating from hereditary spherocytosis.

3. **Cold autoimmune hemolytic anemia (CAIHA or cold hemagglutinin disease)**
   a. RBCs are coated with **IgM and complement** at temperatures below 37°C. RBCs are lysed by complement or phagocytized by macrophages. Antibody is usually anti-I but can be anti-i.
   b. Can be **idiopathic,** or secondary to ***Mycoplasma pneumoniae,*** lymphoma, or infectious mononucleosis
   c. **Laboratory:** Seasonal symptoms; RBC clumping can be seen both macroscopically and microscopically; MCHC >37 g/dL; increased bilirubin, reticulocyte count; positive DAT detects complement-coated RBCs
   d. If antibody titer is high enough, sample must be warmed to 37°C to obtain accurate RBC and indices results.
4. **Paroxysmal cold hemoglobinuria (PCH)**
   a. An **IgG biphasic Donath-Landsteiner antibody** with **P specificity** fixes complement to RBCs in the cold (less than 20°C); the complement-coated RBCs lyse when warmed to 37°C.
   b. Can be **idiopathic,** or secondary to viral infections (e.g., measles, mumps) and non-Hodgkin lymphoma
   c. **Laboratory:** Variable anemia following hemolytic process; increased bilirubin and plasma hemoglobin, decreased haptoglobin; DAT may be positive; Donath-Landsteiner test positive
5. **Hemolytic transfusion reaction**
   a. Recipient has antibodies to antigens on donor RBCs; donor cells are destroyed.
   b. **ABO incompatibility** causes an immediate reaction with massive intravascular hemolysis that is complement induced.
      1) Usually **IgM** antibodies
      2) Can trigger **DIC** due to release of tissue factor from the lysed RBCs
   c. **Laboratory:** Positive DAT, increased plasma hemoglobin
6. **Hemolytic disease of the newborn (HDN)**
   a. **May be** due to **Rh incompatibility** (erythroblastosis fetalis)
      1) Rh negative woman is exposed to Rh antigen from fetus and forms IgG antibody; this antibody will cross the placenta and destroy RBCs of the **next fetus** that is Rh positive.
      2) **Laboratory:** Severe anemia, nRBCs, positive DAT; very high bilirubin levels cause **kernicterus** leading to brain damage
      3) Exchange transfusions *in utero* or shortly after birth
      4) No longer a common problem with use of Rh immunoglobulin (RhoGam)
   b. May be due to **ABO incompatibility**
      1) Group O woman develops IgG antibody that crosses the placenta and **coats** fetal RBCs when fetus is group A or B. The coated RBCs are phagocytized.
      2) **Laboratory:** Mild or no anemia, few spherocytes, weakly positive DAT, slightly increased bilirubin

F. Hemolytic Anemias Due to Extrinsic/Non-Immune Defects

1. All cause a **normocytic/normochromic anemia** caused by trauma to the RBC. All are **acquired** disorders that cause intravascular hemolysis with **schistocytes** and **thrombocytopenia.**
2. **Microangiopathic hemolytic anemias (MAHAs)**
   a. **Disseminated intravascular coagulation (DIC)**
      1) Systemic clotting is initiated by activation of the coagulation cascade due to toxins or conditions that trigger release of procoagulants (tissue factor). Multiple organ failure can occur due to clotting.
      2) Fibrin is deposited in small vessels, causing RBC fragmentation.
   b. **Hemolytic uremic syndrome (HUS)**
      1) Occurs most often in children following a gastrointestinal infection (e.g., *E. coli*)
      2) Clots form, causing **renal damage.**
   c. **Thrombotic thrombocytopenic purpura (TTP)**
      1) **TTP** occurs most often in adults.
      2) It is likely due to a deficiency of the enzyme **ADAMTS 13** that is responsible for breaking down large von Willebrand factor multimers. When multimers are not broken down, clots form, causing RBC fragmentation and central nervous system impairment.
3. **March hemoglobinuria:** Transient hemolytic anemia that occurs after forceful contact of the body with hard surfaces (e.g., marathon runners, tennis players)
4. **Other causes**
   a. Infectious agents (e.g., *P. falciparum, Clostridium perfringens*) damage the RBC membrane. **Schistocytes** and **spherocytes** are seen on the blood smear.
   b. Mechanical trauma, caused by prosthetic heart valves (Waring blender syndrome), chemicals, drugs, and snake venom, damage the RBCs through various mechanisms.
   c. Thermal burns (third degree) cause direct damage to the RBC membrane, producing acute hemolysis, which is characterized by severe anemia with many schistocytes and micro-spherocytes.

X. HEMOGLOBINOPATHIES

A. Introduction: These are a group of inherited disorders causing **structurally abnormal** globin chain synthesis due to **amino acid substitutions** (qualitative defect); changes in RBC deformability and electrophoretic mobility can occur. Homozygous/disease conditions (both globin chains affected) are more serious than heterozygous/trait conditions (only one globin chain affected). **Target cells** are associated with the hemoglobinopathies. Hemoglobin electrophoresis, isoelectric focusing and/or DNA (PCR) analysis may be used to confirm the diagnosis. The

amino acid substitution causing formation of **Hgb S** is the **most common, Hgb C** is the **second** most common, and **Hgb E** is the third most common.

B. Sickle Cell Disease (Hgb SS)

1. **Sickle cell disease** is caused when **valine** replaces **glutamic acid** at position 6 on **both beta chains.** It results in a decrease in hemoglobin solubility and function. Defect is inherited from **both parents.**
2. Occurs most commonly in African-American, African, Mediterranean, and Middle East populations
3. **No Hgb A** is produced, and approximately 80% **Hgb S** and 20% **Hgb F** (the compensatory hemoglobin) are seen. Hgb A_2 is variable.
4. Hemoglobin insolubility results when deoxyhemoglobin is formed. Hemoglobin crystallizes in erythrocytes. It is characterized by the classic **sickled shape** of erythrocytes.
5. **Clinical findings**
   a. Erythrocytes become rigid and trapped in capillaries; blood flow restriction causes lack of oxygen to the tissues, resulting in **tissue necrosis.**
   b. All organs are affected, with **kidney failure** being a common outcome; hyposplenism and joint swelling also occur.
   c. **Vaso-occlusive crisis** occurs with increased bone marrow response to the **hemolytic anemia.** Crisis can be initiated by many physiological factors, including surgery, trauma, pregnancy, high altitudes, etc.
   d. Apparent immunity to *Plasmodium falciparum*
6. Diagnosis is made after 6 months of age (time of beta-gamma globin chain switch), with life expectancy of 50 years with proper treatment. Death usually results from infection or congestive heart failure.
7. **Laboratory**
   a. Severe **normochromic/normocytic hemolytic anemia** with polychromasia resulting from premature release of reticulocytes; bone marrow erythroid hyperplasia (M:E ratio decreases)
   b. Sickle cells, target cells, nucleated RBCs, Pappenheimer bodies, and Howell-Jolly bodies are seen. Increased bilirubin and decreased haptoglobin are characteristic due to hemolysis.
   c. Positive hemoglobin solubility screening test
   d. **Hgb S** migrates with hemoglobins **D and G** on alkaline hemoglobin electrophoresis; can differentiate using acid electrophoresis.

C. Sickle Cell Trait (Hgb AS)

1. **Sickle cell trait** is caused when **valine** replaces **glutamic acid** *at position* 6 on **one beta chain.** Defect is inherited from **one parent.** One normal beta chain can produce some Hgb A.
2. Approximately 60% Hgb A and 40% Hgb S are produced, with normal amounts of Hgbs A_2 and F.

3. This **heterozygous trait** is the most common hemoglobinopathy in the United States.
4. Sickle cell trait generally produces no clinical symptoms. Anemia is rare but, if present, will be normochromic/normocytic, and sickling can occur during rare crisis states (same as in Hgb SS).
5. Positive hemoglobin solubility screening test
6. Apparent immunity to *Plasmodium falciparum*

D. Hgb C Disease/Hgb CC

1. **Hgb C disease** is caused when **lysine** replaces **glutamic acid** at position 6 on **both beta chains.** Defect is inherited from **both parents.**
2. Occurs in the African-American and African populations
3. **No Hgb A** is produced; approximately **90% Hgb C, 2% Hgb A_2,** and **7% Hgb F** are produced. Mild anemia may be present.
4. **Laboratory: Normochromic/normocytic anemia** with target cells; characterized by intracellular rodlike **C crystals**
5. **Hgb C** migrates with hemoglobins **A_2, E, and O** on alkaline hemoglobin electrophresis; can differentiate hemoglobins using acid electrophoresis.
6. The heterozygous Hgb C trait patient is asymptomatic, with no anemia; the one normal beta chain is able to produce approximately 60% Hgb A and 40% Hgb C, with normal amounts of Hgb A_2 and Hgb F.

E. Hgb SC Disease

1. **Hgb SC disease** is a double **heterozygous** condition where an abnormal sickle gene from one parent and an abnormal C gene from the other parent is inherited.
2. Seen in African, Mediterranean, and Middle Eastern populations; symptoms less severe than sickle cell anemia but more severe than Hgb C disease
3. **No Hgb A** is produced; approximately **50% Hgb S** and **50% Hgb C** are produced. Compensatory Hgb F may be elevated up to 7%.
4. **Laboratory:** Moderate to severe **normocytic/normochromic anemia** with target cells; characterized by SC crystals; may see rare sickle cells or C crystals; positive hemoglobin solubility screening test

F. Other Hemoglobinopathies

1. **Hemoglobin E**
   a. Caused when **lysine** replaces **glutamic acid** at position 26 on the **beta chain**
   b. Found more commonly in Southeast Asian, African, and African-American populations
   c. Homozygous condition results in mild anemia with microcytes and target cells; heterozygotes are asymptomatic.
   d. **Hgb E** migrates with hemoglobins **A_2, C,** and **O** on alkaline hemoglobin electrophoresis.

2. **Hemoglobin D**
   a. Caused when **glycine** replaces **glutamic acid** at position 121 on the **beta chain**
   b. Found more commonly in Middle Eastern and Indian populations
   c. Both homozygous and heterozygous conditions are asymptomatic.
   d. **Hgb D migrates** with **Hgb S** and **Hgb G** on alkaline hemoglobin electrophoresis.

XI. THALASSEMIAS

A. Introduction:

Group of inherited disorders causing **decreased rate of synthesis** of a structurally normal globin chain (quantitative defect); characterized by **microcytic/hypochromic** RBCs and **target cells**

1. Classified according to the globin chain affected
2. Found in Mediterranean (beta), Asian (alpha), and African (alpha and beta) populations
3. **Severity varies** from no clinical abnormalities to transfusion-dependent to fatal
4. **Thalassemia major: Severe anemia;** either no alpha or no beta chains produced
5. **Thalassemia minor/trait: Mild anemia;** sufficient alpha and beta chains produced to make normal hemoglobins **A, A_2, and F,** but may be in **abnormal amounts**

B. Beta-Thalassemia

1. **Major/homozygous (Cooley anemia)**
   a. Markedly decreased rate of synthesis or absence of both beta chains results in an excess of alpha chains; no **Hgb A** can be produced; compensate with up to 90% **Hgb F.**
   b. Excess alpha chains precipitate on the RBC membrane, form Heinz bodies, and cause rigidity; destroyed in the bone marrow or removed by the spleen
   c. Symptomatic by 6 months of age; hepatosplenomegaly, stunted growth, jaundice; prominent facial bones, especially the cheek and jaw; iron overload from RBC destruction and multiple transfusions cause organ failure
   d. **Laboratory:** Severe **microcytic/hypochromic anemia,** target cells, teardrops, many nRBCs, basophilic stippling, Howell-Jolly bodies, Pappenheimer bodies, Heinz bodies; increased serum iron and increased bilirubin reflect the hemolysis
2. **Minor/heterozygous**
   a. Decreased rate of synthesis of one of the beta chains; other beta chain normal
   b. **Laboratory:** Mild **microcytic/hypochromic anemia,** with a normal or slightly elevated RBC count; target cells, basophilic stippling
   c. Hgb A is slightly decreased, but **Hgb A_2** is slightly **increased** to compensate

C. Alpha-Thalassemia

1. **Major (hydrops fetalis)**
   a. All **four alpha genes** are **deleted; no normal hemoglobins** are produced.
   b. 80% **hemoglobin Bart's** (γ_4) produced; cannot carry oxygen; incompatible with life; die *in utero* or shortly after birth
2. **Hgb H disease**
   a. **Three alpha genes** are **deleted.** Decrease in alpha chains leads to beta chain excess.
   b. **Hemoglobin H** (β_4), an unstable hemoglobin, is produced. **Heinz bodies** form and rigid RBCs are destroyed in the spleen. Distinguishing characteristics include: moderate **microcytic/hypochromic anemia;** up to 30% Hgb H; the rest is Hgb A.
3. **Minor/trait**
   a. **Two alpha genes** are **deleted.** Patients are usually asymptomatic and discovered accidentally. Up to 6% **Hgb Bart's** in newborns may be helpful in diagnosis; absent by 3 months of age
   b. Mild **microcytic/hypochromic anemia** often with a high RBC count and target cells
4. **Silent carrier**
   a. **One alpha gene** is **deleted.** Patients are asymptomatic and are often not diagnosed unless gene analysis is done.
   b. Borderline low MCV may be the only sign.

D. Thalassemia/Hemoglobinopathy Interactions

1. Caused by the inheritance of a thalassemia gene from one parent and a hemoglobin variant gene from the other parent
2. Severity and symptoms depend on the specific interactions.
3. Common interactions include Hgb S/beta-thalassemia, Hgb C/beta-thalassemia, and Hgb E/beta-thalassemia.

XII. HEMATOLOGY TESTS

A. Blood Cell Enumeration—Manual Methods

1. Manual **WBC count** using a hemacytometer
   a. Dilute a well-mixed, EDTA, whole blood sample 1:20 with 3% glacial acetic acid; allow 10 minutes for complete RBC lysis; fill both sides of hemacytometer; allow 1–2 minutes for settling.
   b. Use bright field or phase microscopy, count WBCs seen in the four 1-mm^2 corner squares on both sides of the hemacytometer, use the 10× objective. Total area counted is **8 mm^2.**
   c. **Formula**

$$\text{WBC/mm}^3 = \frac{\text{Total WBCs counted} \times \text{Dilution (20)}}{\text{Total area counted (mm}^2) \times \text{Depth (0.1)}}$$

d. Alternate dilution factor and area counted can be used; appropriate adjustments must be made to the formula. Other diluents (1% ammonium oxalate) can also be used.

e. **Correction for presence of nucleated RBCs**

$$\text{Corrected WBC/mm}^3 = \frac{100 \times \text{Uncorrected WBCs}}{100 + \text{\# nRBCs per 100 WBCs}}$$

2. **Platelet count**
   a. Dilute a well-mixed, EDTA, whole blood sample 1:100 with 1% ammonium oxalate; allow 10 minutes for complete RBC lysis; fill both sides of hemacytometer; allow 10 minutes for complete settling in a humidified chamber to prevent evaporation.
   b. Use phase (preferred) or bright field microscopy, count platelets seen in the **center** 1-mm^2 square on both sides of the hemacytometer, use the 40× objective. Total area counted is **2 mm^2.**
   c. **Formula**

$$\text{PLT count/mm}^3 = \frac{\text{Total PLTs counted} \times \text{Dilution (100)}}{\text{Total area counted (mm}^2) \times \text{Depth (0.1)}}$$

3. **Sources of error** involving manual cell counts
   a. Specimen clotted
   b. Sample inadequately mixed before diluting
   c. Equipment not thoroughly cleaned or dried
   d. Technical errors due to evaporation on the hemacytometer, diluting/plating, following procedure, counting of cells, calculating results

B. Blood Cell Enumeration—Automated Methods

1. **Electrical impedance**
   a. Cells pass through an aperture with an electrical current flowing through simultaneously. Cells do not conduct current but rather they change electrical resistance, which is then counted as voltage pulses.
   b. The number of pulses generated is proportional to the number of cells present; amplitude of the pulse generated is proportional to the size of the cell.
   c. Sample is diluted in isotonic conductive solution that preserves cell shape and characteristics.
      1) Dilutions used are dependent on instrument/methodology used.
      2) Platelets are counted simultaneously with RBCs.
      3) Sample for counting WBCs is mixed with reagent to lyse RBCs. A commercially available reagent, which both lyses RBCs and converts hemoglobin to cyanmethemoglobin, can be used to determine hemoglobin and WBCs in one dilution.
   d. Thresholds are used to separate cell populations and subpopulations.

e. Hydrodynamic focusing is utilized to reduce **cell coincidence** (chance of one cell being counted more than once).

2. **Light scattering optical method**
   a. Uses a flow cytometer with laser to measure light scattering properties of cells
      1) Forward angle light scatter measures **cell size.**
      2) Side angle light scatter provides information on cell **granularity** and **lobularity.**
      3) Number of pulses generated is proportional to the number of cells present.
   b. Dilutions used are dependent on instrument/methodology used.
3. Interpretative reports give relative percentages and absolute counts for the 5 leukocyte subpopulations (most instruments).
4. Suspect **"flags"** indicate problems: Exceeding linearity, lack of agreement among apertures, unacceptable distribution caused by unusual cell populations.
5. Automated cell count errors
   a. WBC counts exceeding instrument linearity limits result in increased cell turbidity and may falsely increase the hemoglobin, MCH, and MCHC.
   b. Glucose over 600 mg/dL (hyperosmolarity) may increase the MCV and hematocrit and decrease the MCHC.
   c. Cold agglutinins increase the MCV, MCH, and MCHC and decrease the RBC count and hematocrit.
   d. Lipemia increases the hemoglobin, MCH, and MCHC.
   e. Repeat the analysis if:
      1) **Rule of three** (shown below) failure on a normocytic sample (especially MCHC >37 g/dL)
         a) RBC $\times$ 3 = Hgb
         b) RBC $\times$ 9 = Hct
         c) Hgb $\times$ 3 = Hct
      2) Any result outside linearity limits established by manufacturer (dilute into linearity range)
      3) Unexplained **delta check failures** (e.g., results do not correlate with recent previous results, especially MCV)

C. Histograms and Scatterplots

1. A **histogram** utilizes **impedance technology,** and it is a representation of cell number versus one measured property, usually cell size. It is used for WBCs, RBCs, and platelets.
   a. **WBC histogram**
      1) 35–450 fL is the reference size range for **WBCs.**
      2) 1st peak: 35–90 fL is the range for **lymphocytes.**
      3) 2nd peak: 90–160 fL is the range for **mononuclear cells (monocytes, reactive lymphocytes,** and **immature WBCs).**
      4) 3rd peak: 160–450 fL is the range for **granulocytes.**

b. **Abnormal WBC histogram**
   1) Population before 35 fL may indicate nucleated RBCs **(nRBCs), giant** or **clumped platelets.**
   2) Peak overlap at 90 fL may indicate **reactive lymphocytes** or **blast cells.**
   3) Peak overlap at 160 fL may indicate an increase in **bands, immature neutrophils, eosinophils,** or **basophils.**
   4) Population after 450 fL may indicate a **high granulocyte** count.

c. **RBC histogram**
   1) 36 fL and above is the reference size range for **RBCs.**
   2) A normal RBC histogram will show a single peak between 70 and 110 fL that will correlate with the MCV.

d. **Abnormal RBC histogram**
   1) Two peaks indicate a **dimorphic** erythrocyte population.
   2) Increased curve width will correlate with an increased RDW **(anisocytosis).**
   3) Shift to the right indicates an increased MCV **(macrocytic).**
   4) Shift to the left indicates a decreased MCV **(microcytic).**

e. **Platelet histogram**
   1) 2–20 fL is the reference size range for **platelets.**
   2) **Lower region interference** (<2 fL) indicates electrical interference; **upper region interference** (>20 fL) indicates microcytic RBCs or schistocytes, giant or clumped platelets.

2. A **scatterplot/scattergram** is a two-dimensional representation of two or more cell properties or characteristics plotted against each other (e.g., size versus granularity or lobularity). Scatterplots of WBCs are displayed on a monitor and are color coded for different subpopulations.
   a. Methodologies include radio frequency, fluorescence, and cytochemistry.
   b. Correlation between abnormal cell populations and suspect flags is generally very good.

D. Hemoglobin Measurement

1. Blood oxygen capacity: Measures functional hemoglobin

$$\frac{\text{Oxygen capacity in mL/dL blood}}{1.34 \text{ (Hgb oxygen capacity)}} = \text{grams of Hgb/dL blood}$$

2. Cyanmethemoglobin method is the **reference method;** it will measure all hemoglobins except for sulfhemoglobin.
   a. Uses **Drabkin** reagent (potassium ferricyanide and KCN) to lyse RBCs and convert heme iron to the ferric state (Fe^{3+}), forming methemoglobin. KCN in the reagent converts methemoglobin to cyanmethemoglobin; read spectrophotometrically at **540 nm.**
   b. Automated cell counters use some modification of the cyanmethemoglobin method to determine hemoglobin concentration.

E. Reticulocyte Counts

1. **Supravital new methylene blue stain** is used to demonstrate reticulum in reticulocytes.
2. Reticulocyte (retic) formulas:
   a. Relative count

$$\text{Retics (\%)} = \frac{\text{\# of Retics}}{\text{1000 RBCs observed}} \times 100$$

   b. Absolute count

$$\text{Absolute retic } (\times 10^9/\text{L}) = \text{Retic \%} \times \text{RBC count } (\times 10^{12}/\text{L})/100$$

3. Corrected reticulocyte counts are calculated to account for the degree of anemia by using a standard normal hematocrit of 45% expressed in SI units.

$$\text{Corrected retic count} = \text{Retic \%} \times \frac{\text{Hct (L/L)}}{0.45\ \text{L/L}}$$

4. Immature reticulocyte fraction (IRF) is an instrument calculated parameter that indicates the ratio of immature reticulocytes to total reticulocytes.

F. Erythrocyte Sedimentation Rate: ESR measures degree of settling of RBCs in plasma in an anticoagulated specimen during a specific time, usually 1 hour. High fibrinogen or protein levels increase the ESR.

1. **Reference range:** Approximately 0–20 mm/hr; age and sex dependent
2. ESR is **increased** in chronic inflammatory conditions, including rheumatoid arthritis and pregnancy (increased fibrinogen), bacterial infection, malignancy, tissue damage, multiple myeloma, Waldenström macroglobulinemia, and severe anemia.
   a. **Sources of error** causing **falsely increased** results: Tilted column, hemolysis, increased room temperature
3. ESR is **normal to decreased** in polycythemia, sickle cell anemia, spherocytosis, and other conditions with poikilocytosis (prevents rouleaux formation).
   a. **Sources of error** causing **falsely decreased** results: Clotted sample, excess anticoagulant, “old” blood (spherocytes form)

G. Hemoglobin F (Kleihauer-Betke method): Count dense-staining Hgb F cells and the number of ghost cells containing Hgb A to obtain percentage.

1. It is used to detect the presence of fetal cells in the maternal circulation during problem pregnancies because Hgb F in fetal cells resists acid elution.
2. It differentiates hereditary persistence of fetal hemoglobin from other conditions associated with high Hgb F levels.
3. Normal newborns have 70–90% Hgb F levels.

H. Solubility Test for Hemoglobin S (Sickle Cell Prep)

1. Hemoglobin S is insoluble when combined with a reducing agent (sodium dithionite).
2. Hgb S will crystallize and give a turbid appearance to the solution.
3. The test will **not** differentiate homozygous from heterozygous conditions containing Hgb S.
4. Follow up a positive solubility test with hemoglobin electrophoresis.

I. Hemoglobin Electrophoresis

1. Procedure for the identification of normal and abnormal hemoglobins
2. Methodology is based on net negative charges, which cause hemoglobins to migrate from the negative **(cathode)** region toward the positive **(anode)** region. The distance a particular hemoglobin molecule migrates is due to its net electrical charge.
3. Two types of electrophoresis: **Cellulose acetate** at **pH 8.6** and **citrate agar** at **pH 6.2**
4. Migration of hemoglobin is dependent on net negative charge and buffer pH.
5. **Cellulose Acetate (pH 8.6) Hemoglobin Electrophoresis**

Cathode (–)					Anode (+)
(x) origin	**A_2**	**S**	**F**	**A**	
	C	**D**			
	E	**G**			
	O				

   a. At pH 8.6, Hgb A migrates the fastest, and Hgb A_2, C, E, and O migrate the slowest.

6. **Citrate Agar (pH 6.2) Hemoglobin Electrophoresis**

Anode (+)						Cathode (–)
	C	**S**	(x) origin	**A**	**F**	
				A_2		
				D		
				G		
				E		
				O		

a. At pH 6.2, Hgb S is differentiated from Hgb D and G.
b. At pH 6.2, Hgb C is differentiated from Hgb A_2, E, and O.

J. Flow Cytometry

1. **Principle: Cells** in a suspension of buffered solution are **labeled with** one to several **fluorescent compounds.** This cell suspension is run under high pressure and in a single, narrow stream through a laser, causing excitation of the fluorescent compound(s) and resulting in the emission of light energy. This energy is detected by a photomultiplier tube and is subsequently converted into computerized data, which upon analysis provides information regarding number, size, and cellular composition of the population assayed.
2. Major components of a **flow cytometer**
   a. **Fluidics**—Flow chamber for single cell separation, sheath fluid, and hydrodynamic focusing
   b. **Optics**—Excitation light sources include lasers (argon, krypton, helium-neon, helium-cadmium, diode) or lamps (mercury, xenon-mercury). Light is separated by dichroic mirrors and filters.
   c. **Electronics**—Photomultiplier tube detects light energy, then coverts this to voltage pulses; computers translate pulses into data files.
3. Hydrodynamic focusing uses laminar flow to line the cells up single file.
4. **Light** is **scattered** at 90 degrees or forward.
5. **Fluorescent dyes** used in flow cytometry include, but are not limited to, allophycocyanin (APC), acridine orange (AO), chromomycin A3, cyanine dye (Cy), fluorescein isothiocyanate (FITC), peridinin chlorophyll protein (PerCP), phycoerythrin (PE), propidium iodine (PI), pyronin Y, rhodamine isothiocyanate, and sulforhodamine 101 acid chloride.
6. **Specimens analyzed** by flow cytometry: Leukocytes, erythrocytes, lymph nodes, peripheral whole blood, bone marrow, tumors, and other tissues
7. **Clinical applications:** Differentiation of T and B cells; cell cycle analysis; diagnosing and following patients with leukemia, lymphoma, and autoimmune or deficiency diseases; karyotyping; and monitoring a patient's response to drug therapy

review questions

INSTRUCTIONS Each of the questions or incomplete statements that follow is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

Hematopoiesis

1. What is the first type of cell produced by the developing embryo?
   A. Erythrocyte
   B. Granulocyte
   C. Lymphocyte
   D. Thrombocyte

2. What percentage of tissue located in the bone marrow cavities of adults is fat?
   A. 10%
   B. 25%
   C. 50%
   D. 75%

3. Which of the following is *not* characteristic of pluripotent hematopoietic stem cells?
   A. Possess self-renewal ability
   B. Produce progenitor cells committed to a single cell lineage
   C. Express the stem cell marker CD13
   D. Are morphologically unrecognizable

4. In an adult, what are the two best areas for obtaining active bone marrow by aspiration?
   A. Vertebra, tibia
   B. Sternum, vertebra
   C. Anterior iliac crest, tibia
   D. Posterior iliac crest, sternum

5. What is the normal ratio of myeloid to erythroid precursors in bone marrow (M:E ratio)?
   A. 1:1
   B. 1:3
   C. 4:1
   D. 8:1

6. Which of the following does *not* accurately describe hematopoietic growth factors?
   A. Bind to target cell receptors to express activity
   B. Action of majority is lineage restricted
   C. May promote or suppress cell death
   D. Can stimulate or inhibit cell proliferation

7. In the third month of gestation, what is the primary site of hematopoiesis?
   A. Liver
   B. Marrow of long bones
   C. Spleen
   D. Yolk sac

8. The mechanism that relays information about tissue oxygen levels to erythropoietin-producing sites is located in the
   A. Brain
   B. Kidney
   C. Liver
   D. Spleen

9. Antigen-independent lymphopoiesis occurs in primary lymphoid tissue located in the
   A. Liver and kidney
   B. Spleen and lymph nodes
   C. Peyer's patches and spleen
   D. Thymus and bone marrow

10. Programmed cell death is called
    A. Necrosis
    B. Apoptosis
    C. Cellular senescence
    D. Terminal differentiation

11. In what area of the bone marrow does hematopoiesis take place?
    A. Cords
    B. Endosteum
    C. Endothelium
    D. Sinuses

12. Bone marrow cellularity refers to the ratio of
    A. Red cell precursors to white cell precursors
    B. Hematopoietic tissue to adipose tissue
    C. Granulocytic cells to erythrocytic cells
    D. Extravascular tissue to intravascular tissue

13. Interleukins and colony stimulating factors are cytokines produced by
    A. B lymphocytes and erythrocytes
    B. Erythrocytes and thrombocytes
    C. Monocytes and T lymphocytes
    D. Neutrophils and monocytes

14. What is the approximate total blood volume in an adult?
    A. 1 L
    B. 2 L
    C. 6 L
    D. 12 L

15. The myeloid progenitor cell can produce cells committed to
    A. Granulocytic, erythrocytic, monocytic, or megakaryocytic lineages
    B. Granulocytic, monocytic, lymphocytic, or megakaryocytic lineages
    C. Erythrocytic, granulocytic, monocytic, or lymphocytic lineages
    D. Erythrocytic, granulocytic, lymphocytic, or megakaryocytic lineages

16. The largest hematopoietic cells in normal bone marrow are
    A. Osteoblasts
    B. Osteoclasts
    C. Megakaryocytes
    D. Plasma cells

17. When evaluating a bone marrow aspirate smear, which finding is considered abnormal?
    A. A predominance of granulocyte precursors as compared to nucleated red cells
    B. Detection of stainable iron in macrophages and erythroid precursors with Prussian blue
    C. An average of three megakaryocytes seen per low power (10×) field
    D. The presence of 10% myeloblasts on the cell differential count

18. As most blood cell lines mature, which of the following is characteristic?
    A. Cell diameter increases
    B. Nucleus to cytoplasm ratio (N:C) decreases
    C. Nuclear chromatin becomes less condensed
    D. Basophilia of the cytoplasm increases

19. Which of the following describes thrombopoietin (TPO)?
    A. Renal hormone that regulates marrow red cell production
    B. Marrow hormone secreted by developing megakaryoblasts
    C. Hormone produced by the liver that stimulates megakaryopoiesis
    D. Pituitary hormone that controls platelet sequestration by the spleen

20. When the hepatic phase of fetal life is reactivated in an adult, hematopoiesis can be termed
    A. Myeloid or medullary
    B. Myeloid metaplasia or extramedullary
    C. Myelophthisis or myelodysplasia
    D. Mesoblastic or mesenchymal

Erythrocytes

21. What is the average life span of a normal red blood cell?
    A. 1 day
    B. 10 days
    C. 60 days
    D. 120 days

22. The Na^+–K^+ cation pump is an important mechanism in keeping the red blood cell intact. Its function is to maintain a high level of
    A. Intracellular Na^+
    B. Intracellular K^+
    C. Plasma Na^+
    D. Plasma K^+

23. Which of the following depicts the structure of the hemoglobin molecule?
    A. Two heme groups, two globin chains
    B. Four heme groups, two globin chains
    C. Two heme groups, four globin chains
    D. Four heme groups, four globin chains

24. Which of the following describes the process known as *culling?*
    A. Release of red cells from the bone marrow
    B. Binding of free hemoglobin by transport proteins
    C. Incorporation of iron into protoporphyrin IX
    D. Removal of abnormal red cells by the spleen

25. Hemoglobin forms that are incapable of oxygen transport include
    A. Deoxyhemoglobin and oxyhemoglobin
    B. Oxyhemoglobin and carboxyhemoglobin
    C. Carboxyhemoglobin and methemoglobin
    D. Methemoglobin and deoxyhemoglobin

26. The majority of iron found in an adult is a constituent of
    A. Ferritin
    B. Myoglobin
    C. Hemoglobin
    D. Peroxidase

27. A *senescent* red blood cell is one that has
    A. Been hemolyzed
    B. Lived its life span
    C. Become deformed
    D. Lost its mitochondria

28. What red cell morphologic abnormality is described by the term "poikilocytosis"?
    A. Variations in size
    B. Deviations from normal shape
    C. Presence of inclusions
    D. Alterations in hemoglobin concentration

29. Howell-Jolly bodies are composed of
    A. DNA
    B. Iron
    C. Reticulum
    D. RNA

30. When spherocytes are reported, what is observed on the peripheral blood smear?
    A. Red cells without a central pallor
    B. Red cells with blunt projections
    C. Red cells with sharp projections
    D. Red cells with intracellular rod-shaped crystals

31. The red cells found in lead poisoning characteristically exhibit coarse granules composed of ______________ that are reported as ______________.
    A. Precipitated hemoglobin; Pappenheimer bodies
    B. Aggregated ribosomes; basophilic stippling
    C. Nuclear fragments; Pappenheimer bodies
    D. Excess iron deposits; basophilic stippling

32. Rouleaux of red blood cells when seen in the monolayer of a blood smear is characteristic of
    A. Hypersplenism
    B. Hypogammaglobulinemia
    C. Cold hemagglutinin disease
    D. Multiple myeloma

33. Which of the following is most frequently associated with the inclusion bodies seen in Color Plate 1■?
    A. Iron overload state
    B. Post-transfusion
    C. Post-splenectomy
    D. Iron-deficient state

34. Which of the following statements about iron absorption is *true?*
    A. Absorption occurs in the ileum.
    B. The mucosal cell always absorbs the correct amount of iron to meet needs.
    C. Absorption increases when erythropoietic activity increases.
    D. Alkaline pH favors absorption.

35. What term describes a mature red blood cell that contains iron granules or deposits?
    A. Siderosome
    B. Sideroblast
    C. Ringed sideroblast
    D. Siderocyte

36. Which of the following is associated with a "shift to the left" in the oxygen dissociation curve of hemoglobin?
    A. Decreased pH and elevated temperature
    B. Decreased oxygen affinity
    C. Decreased oxygen release
    D. Presence of 2,3-bisphosphoglycerate (2,3-BPG)

37. Which of the following statements does *not* characterize erythropoietin (EPO)?
    A. Transforms the CFU-E into the earliest recognizable RBC precursor
    B. Increases the rate of red blood cell production by the bone marrow
    C. Shortens the maturation time of developing erythroid precursors
    D. Decreases stimulation of erythropoiesis when cellular hypoxia increases

38. Which of the following factors will result in an immediate increase in oxygen delivery to the tissues?
    A. Increased pH
    B. High altitudes
    C. Increased hemoglobin binding of 2,3-BPG
    D. Increased renal release of erythropoietin

39. Periods of intense erythropoietin activity cause premature release of marrow reticulocytes into the blood. Which of the following is *not* true of these early reticulocytes?
   A. Loss of residual RNA occurs immediately upon marrow release
   B. Circulate longer than usual before reaching maturity
   C. May be termed shift or stress reticulocytes
   D. Show diffuse basophilia with Wright's stain

40. Which of the following inclusions is *only* visible with supravital staining?
   A. Basophilic stippling
   B. Cabot rings
   C. Heinz bodies
   D. Pappenheimer bodies

41. The presence of schistocytes on the peripheral blood smear is commonly associated with
   A. Increased iron mobilization
   B. Increased red cell destruction
   C. Decreased erythropoietin activity
   D. Decreased red cell proliferation

42. Which of the following may be a sign of accelerated bone marrow erythropoiesis?
   A. Hypercellular marrow with a decreased number of RBC precursors
   B. Bone marrow M:E ratio of 6:1
   C. Nucleated red cells in the peripheral circulation
   D. Low erythrocyte, hemoglobin, and hematocrit levels

43. Microcytic, hypochromic red cells are most often associated with impaired
   A. DNA synthesis
   B. RNA metabolism
   C. Hemoglobin synthesis
   D. Enzyme metabolism

44. When in bone marrow, the nucleated red cells present in Color Plate 2■ would be staged as
   A. Basophilic normoblasts
   B. Polychromatophilic normoblasts
   C. Orthochromic normoblasts
   D. Pronormoblasts

45. When acanthocytes are found on the blood smear, it is usually the result of
   A. Abnormal membrane permeability
   B. Altered membrane lipids
   C. Mechanical trauma
   D. Polymerization of hemoglobin molecules

46. Which erythrocyte metabolic pathway generates adenosine triphosphate (ATP) via glycolysis?
   A. Embden-Meyerhof
   B. Hexose monophosphate
   C. Rapoport-Luebering
   D. Methemoglobin reductase

47. Which of the following red blood cell precursors is the last stage to undergo mitosis?
   A. Pronormoblast
   B. Basophilic normoblast
   C. Polychromatophilic normoblast
   D. Orthochromic normoblast

48. The major adult hemoglobin requires the synthesis of alpha-globin chains and
   A. Beta-globin chains
   B. Delta-globin chains
   C. Epsilon-globin chains
   D. Gamma-globin chains

49. Defective nuclear maturation commonly results in the production of red cells that are
   A. Normocytic
   B. Hypochromic
   C. Macrocytic
   D. Microcytic

50. The major storage form of iron is
    A. Ferritin
    B. Transferrin
    C. Hemosiderin
    D. Hemachromatin

51. The red cells observed on a peripheral blood smear show extreme anisocytosis with an equal number of macrocytes and microcytes. Which of the following values correlate with this finding?
    A. MCV 108.0 fL, RDW 14.0%
    B. MCV 90.0 fL, RDW 25.0%
    C. MCV 75.0 fL, RDW 16.0%
    D. MCV 88.0 fL, RDW 12.0%

52. Excessive extravascular red cell destruction is associated with
    A. Hemoglobinemia
    B. Bilirubinemia
    C. Hemoglobinuria
    D. Hemosiderinuria

53. Which protein is primarily responsible for transport of hemoglobin dimers resulting from intravascular hemolysis?
    A. Hemopexin
    B. Albumin
    C. Hemosiderin
    D. Haptoglobin

54. The morphologic abnormality characteristically found in hemoglobinopathies is
    A. Elliptocytes
    B. Dacryocytes
    C. Codocytes
    D. Discocytes

55. Where do the early and late stages of heme synthesis occur?
    A. On ribosomes
    B. In mitochondria
    C. In cytoplasm
    D. In nucleoli

56. Spectrin is a protein that occupies a major role in
    A. Red cell membrane structure
    B. Reducing ferric iron
    C. Red cell transport and removal of CO_2
    D. Iron recovery during hemoglobin degradation

57. What is the function of reduced glutathione (GSH) in the red blood cell?
    A. Promotes Kreb's cycle activity
    B. Maintains anion balance during the "chloride shift"
    C. Neutralizes intracellular oxidants that accumulate
    D. Prevents oxygen uptake by hemoglobin

58. What does measuring the total iron-binding capacity (TIBC) represent?
    A. Amount of free iron in serum
    B. Circulating protein-bound iron
    C. Amount of iron that transferrin can bind
    D. Indirect measurement of iron stores

59. Serum ferritin is a good indicator of the amount of
    A. Cytochrome iron
    B. Storage iron
    C. Hemoglobin iron
    D. Transferrin saturation

60. Fetal hemoglobin differs from adult hemoglobin in that hemoglobin F
    A. Has a lower oxygen affinity
    B. Resists elution from red cells with acid solutions
    C. Is no longer synthesized after birth in a normal individual
    D. Has four gamma-globin chains

Erythrocyte Disorders

61. Impaired DNA metabolism is characteristic of
    A. Hemoglobin C disease
    B. Iron-deficiency anemia
    C. Sideroblastic anemia
    D. Megaloblastic anemia

62. Which of the following is associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency?
    A. G6PD gene is located on the X chromosome.
    B. Ongoing intravascular hemolysis occurs.
    C. All circulating red cells, including reticulocytes, lack enzyme activity.
    D. Splenectomy can relieve the rate of red cell destruction.

63. In regard to variant hemoglobin E, $\alpha_2 \beta_2{}^{26\ \mathrm{Glu} \rightarrow \mathrm{Lys}}$, which of the following statements is *false?*
    A. There are two normal alpha chains.
    B. Glutamic acid replaces lysine on position 26 of the beta chains.
    C. Hemoglobin E is the second most common hemoglobin variant known.
    D. Glutamic acid is normally found at position 26 of the beta chain.

64. Color Plate 3■ shows the peripheral blood of a 16-year-old female with a sporadic history of dizzy spells, fainting, and jaundice. This patient also had a history of periodic abdominal pain related to gallstones. Upon physical examination, she exhibited mild splenomegaly. Her hemoglobin was 107 g/L (10.7 g/dL), hematocrit was 0.32 L/L (32%), red cell indices were normal, and the direct antiglobulin test was negative. Based on history and peripheral blood morphology, which of the following statements is most likely *true?*
    A. Hemoglobin S will be revealed by electrophoresis.
    B. Tests to confirm iron deficiency should be ordered.
    C. An intrinsic hereditary defect of red cells should be suspected.
    D. The anemia is secondary to spleen and gallbladder disorders.

65. A 9-month-old male was seen in the Emergency Department with a femur fracture that had occurred from a fall down the stairs. Upon physical examination, the physician noted hepatosplenomegaly, extreme pallor, and a slight arrhythmia. A complete blood count revealed the following:

WBC	12.2×10^9/L ($12.2 \times 10^3/\mu$L)
RBC	3.05×10^{12}/L ($3.05 \times 10^6/\mu$L)
Hemoglobin	61 g/L (6.1 g/dL)
Hematocrit	0.20 L/L (20%)
MCV	65.5 fL
MCH	20 pg
MCHC	305 g/L (30.5 g/dL)
RDW	25%

The Wright's stained blood smear showed the findings seen in Color Plate 4■. Hemoglobin electrophoresis was ordered with results as follows:

Hgb A	0%
Hgb A_2	3%
Hgb F	97%

Which condition is most likely causing the hematologic abnormalities?
    A. Alpha-thalassemia major
    B. Cooley beta-thalassemia major
    C. Hemoglobin H disease
    D. Hereditary persistence of hemoglobin F

66. A 14-year-old African-American male was seen in the clinic for abdominal pain. A complete blood count revealed the following:

WBC	7.0×10^9/L (7.0×10^3/µL)
RBC	2.90×10^{12}/L (2.90×10^6/µL)
Hemoglobin	85 g/L (8.5 g/dL)
Hematocrit	0.25 L/L (25%)
MCV	86.2 fL
MCH	29.3 pg
MCHC	340 g/L (34.0 g/dL)
RDW	21%

The peripheral smear showed the red blood cell morphology seen in Color Plate 5■. What condition is suggested by these findings?
A. Hemoglobin E disease
B. Hemoglobin S disease
C. Hemoglobin SC disease
D. Hemoglobin C disease

67. Pica is most commonly associated with which of the following conditions?
A. Pyridoxine deficiency
B. Lack of erythrocyte folate
C. Iron deficiency
D. Porphyrias

68. Of the following, the leading cause of folate deficiency is
A. Increased requirements
B. Dietary insufficiency
C. Drug inhibition
D. Malabsorption

69. Which of the following statements about sickle cell syndromes is *false?*
A. Asplenism may result from repeated sickling crises in the homozygous state.
B. Heterozygous persons may be partly protected from infection by falciparum malaria.
C. Hemoglobin S is more soluble in dithionite than is normal hemoglobin.
D. Trait conditions are generally asymptomatic with no sickle cell formation.

70. The findings seen in Color Plate 6■ can be found in patients with microangiopathic hemolytic anemia (MAHA). Which of the following conditions could *not* be responsible for this type of red cell destruction?
A. Disseminated intravascular coagulation (DIC)
B. Hemolytic uremic syndrome (HUS)
C. Thrombotic thrombocytopenic purpura (TTP)
D. Idiopathic thrombocytopenic purpura (ITP)

71. Which of the following blood findings does *not* correlate with the presence of ringed sideroblasts in the bone marrow?
A. Pappenheimer bodies
B. Basophilic stippling
C. Increased total iron-binding capacity
D. Increased percent transferrin saturation

72. Which of the following conditions is *not* usually associated with marked reticulocytosis?
A. Four days after a major hemorrhage
B. Drug-induced autoimmune hemolytic anemia
C. Sickle cell anemia
D. Pernicious anemia

73. Hereditary stomatocytosis is manifested physiologically by changes in
    A. Hemoglobin oxygen affinity
    B. Membrane cation permeability
    C. Efficiency of hemoglobin reduction
    D. Glycolytic ATP production

74. In addition to an increase in red blood cells, which of the following is characteristic of polycythemia vera?
    A. Decreased platelets, decreased granulocytes, decreased erythropoietin level
    B. Decreased platelets, decreased granulocytes, increased erythropoietin level
    C. Increased platelets, increased granulocytes, increased erythropoietin level
    D. Increased platelets, increased granulocytes, decreased erythropoietin level

75. Which of the following is *not* characteristic of aplastic anemia?
    A. Extramedullary hematopoiesis
    B. Bone marrow hypoplasia
    C. Absolute reticulocytopenia
    D. Blood findings of pancytopenia

76. What values would you expect to obtain on hemoglobin and hematocrit determinations done immediately after a major hemorrhage, if hemoglobin and hematocrit values were normal prior to the hemorrhage?
    A. Both normal
    B. Both decreased
    C. Hemoglobin decreased, hematocrit normal
    D. Hemoglobin normal, hematocrit decreased

77. Results from a 1-day-old infant include a hemoglobin of 201 g/L (20.1 g/dL), hematocrit of 0.60 L/L (60.0%), MCV of 110.2 fL, and 4 nucleated red cells/100 WBCs. How should these results be interpreted?
    A. The elevated hemoglobin and hematocrit values indicate possible dehydration.
    B. The nucleated red cells suggest accelerated erythropoiesis due to a hemolytic process.
    C. Testing should be done to identify the cause of the macrocytosis.
    D. No further testing is indicated.

78. When viewing Color Plate 7■, the red blood cells with a single elongated projection are known as ______________ and may be seen in ______________
    A. Acanthocytes; liver disease
    B. Echinocytes; liver disease
    C. Drepanocytes; myelofibrosis
    D. Dacryocytes; myelofibrosis

79. A patient with normocytic, normochromic anemia secondary to small cell carcinoma may be exhibiting an anemia designated as
    A. Hemolytic
    B. Megaloblastic
    C. Myelophthisic
    D. Sideroblastic

80. Idiopathic aplastic anemia is best defined as a form of anemia that
    A. Has no identifiable cause
    B. Is caused by a physician's treatment
    C. Follows exposure to ionizing radiation
    D. Develops after a viral infection

81. Which of the following is a true red blood cell aplasia?
    A. Marrow replacement anemia
    B. Fanconi anemia
    C. Diamond-Blackfan anemia
    D. Donath-Landsteiner anemia

82. Which of the following is *not* a cause of absolute secondary erythrocytosis?
   A. Defective cardiac or pulmonary function
   B. High-altitude adjustment
   C. Dehydration secondary to diuretic use
   D. Hemoglobins with increased oxygen affinity

83. A cellulose acetate hemoglobin electrophoresis (alkaline pH), performed on the blood of a stillborn infant, revealed a single band that migrated farther toward the anode than did the Hb A control. What is the most likely composition of the stillborn infant's hemoglobin?
   A. Four beta chains
   B. Four gamma chains
   C. Two alpha and two beta chains
   D. Two alpha and two gamma chains

84. The most likely cause of the stillborn infant's condition in question 83 is
   A. Erythroblastosis fetalis
   B. Rh hemolytic disease of the fetus
   C. Hydrops fetalis
   D. ABO hemolytic disease of the newborn

85. Which of the following conditions show similar CBC and blood smear findings?
   A. Beta-thalassemia major and minor
   B. Folic acid and vitamin B_{12} deficiencies
   C. Acute and chronic blood loss
   D. Sickle cell disease and trait

86. Which of the following would be useful in identifying the cause of the blood profile seen in Color Plate 8■?
   A. Osmotic fragility test
   B. Reticulocyte count
   C. Direct antiglobulin test
   D. Urine urobilinogen level

87. Which of the following conditions is *not* associated with the presence of schistocytes and spherocytes?
   A. Clostridial septicemia
   B. Prosthetic heart valves
   C. Severe thermal burns
   D. Aplastic anemia

88. A 30-year-old woman who has been vomiting for 3 days has a hemoglobin value of 180 g/L (18.0 g/dL) and a hematocrit of 0.54 L/L (54.0%). Her results suggest the presence of
   A. Absolute erythrocytosis
   B. Primary polycythemia
   C. Secondary polycythemia
   D. Relative polycythemia

89. An excessive accumulation of iron in body tissues is called
   A. Hemochromatosis
   B. Erythroblastosis
   C. Megaloblastosis
   D. Acrocyanosis

90. Abetalipoproteinemia is characterized by mild anemia and numerous ________________ on the peripheral blood smear.
   A. Acanthocytes
   B. Elliptocytes
   C. Echinocytes
   D. Stomatocytes

91. What is the most common cause of iron deficiency?
   A. Bleeding
   B. Gastrectomy
   C. Inadequate diet
   D. Intestinal malabsorption

92. Which of the following does *not* characterize beta-thalassemia major?
   A. Transfusion-dependent anemia
   B. Decreased alpha chains result in excess beta chains.
   C. Iron chelation therapy is necessary.
   D. Common in persons of Mediterranean ancestry

93. In the anemia of chronic disease, what are the usual serum iron and transferrin levels?
   A. Serum iron decreased, transferrin decreased
   B. Serum iron decreased, transferrin increased
   C. Serum iron normal, transferrin normal
   D. Serum iron increased, transferrin increased

94. In children, the most important effect of lead poisoning is on the
   A. Liver
   B. Kidney
   C. Neurologic system
   D. Development of erythrocytes

95. Which of the following would *not* result in the dual population of red cells represented in Color Plate 9■?
   A. Blood transfusion
   B. Oral iron therapy
   C. Spleen removal
   D. Coexisting deficiencies

96. What is the most likely genetic defect in the hemoglobin of cells seen in Color Plate 10■?
   A. Substitution of valine for glutamic acid in position 6 of the alpha-globin chain
   B. Substitution of valine for glutamic acid in position 6 of the beta-globin chain
   C. Substitution of lysine for glutamic acid in position 6 of the alpha-globin chain
   D. Substitution of lysine for glutamic acid in position 6 of the beta-globin chain

97. On what is the classification of sickle cell trait versus sickle cell disease based?
   A. Severity of the clinical symptoms
   B. Number of irreversibly sickled cells (ISCs)
   C. Level of compensatory hemoglobin F
   D. Percentage of hemoglobin S on electrophoresis

98. Which of the following is the most appropriate treatment for sickle cell anemia?
   A. Hydroxyurea
   B. Supportive therapy
   C. Hyperbaric oxygen
   D. Iron

99. Which of the following values can be used to indicate the presence of a hemolytic anemia?
   A. Hemoglobin level
   B. Hematocrit level
   C. Erythrocyte count
   D. Reticulocyte count

100. A pre-operative, 20-year-old female has a mild microcytic anemia, with target cells and stippled red cells observed on the blood smear. Her hemoglobin A_2 level is quantified at 5%. What do these findings suggest?
   A. Iron-deficiency anemia
   B. Heterozygous alpha-thalassemia
   C. Heterozygous beta-thalassemia
   D. Hemoglobin S/beta-thalassemia

101. What causes the hemolytic process in glucose-6-phosphate dehydrogenase deficiency following oxidant exposure?
   A. Coating of red cells by antibody
   B. Osmotic pressure changes
   C. Complement attachment
   D. Precipitation of denatured hemoglobin

102. In clinically severe hereditary spherocytosis, which of the following findings would *not* be found post-splenectomy?
   A. Rise in the red cell count and hemoglobin level
   B. Higher number of circulating reticulocytes
   C. Increased number of Howell-Jolly bodies
   D. Transient elevation in the platelet count

103. Which of the following laboratory results is *not* consistent with accelerated red cell destruction?
   A. Increased serum bilirubin
   B. Increased plasma hemoglobin
   C. Increased serum lactate dehydrogenase (LD)
   D. Increased serum haptoglobin

104. Acquired hemolytic anemias are usually due to
   A. Extracorpuscular factors
   B. Defects within the bone marrow
   C. Intracellular factors
   D. Changes in hemoglobin stability

105. The antibody associated with paroxysmal cold hemoglobinuria shows specificity for
   A. ABO antigens
   B. I antigens
   C. P antigens
   D. Rh antigens

106. A 69-year-old male is admitted with pallor, mild tachycardia, and difficulty walking because of numbness in the extremities. His CBC reveals a hemoglobin of 78 g/L (7.8 g/dL), a hematocrit of 0.25 L/L (25.0%), and MCV of 118.5 fL. This patient's symptoms and the blood findings seen in Color Plate 11■ are most suggestive of anemia due to a lack of
   A. Folic acid
   B. Vitamin B_{12}
   C. Vitamin B_6
   D. Ascorbic acid

107. A clinical laboratory scientist examined a Wright's stained peripheral smear and saw what appeared to be small, dark-staining granules in the mature erythrocytes. A second smear was stained with Prussian blue and a positive result was obtained. Based on this information, which of the following would you expect to be abnormal?
   A. Plasma hemoglobin level
   B. Serum ferritin level
   C. Hemoglobin electrophoresis
   D. Test for parietal cell antibodies

108. Hemoglobinopathies are characterized by
   A. Absent or reduced rate of globin-chain synthesis
   B. Inability to transport and release oxygen to the tissues
   C. Inhibition of iron chelation needed for heme biosynthesis
   D. Production of structurally abnormal hemoglobin variants

109. Which of the following statements about hereditary spherocytosis is *true?*
   A. Abnormally shaped cells are produced in the bone marrow.
   B. Cells have a decreased mean cell hemoglobin concentration (MCHC).
   C. Membrane loss and red cell trapping occur in the splenic microcirculation.
   D. Red cell osmotic fragility is decreased.

110. Which of the following statements about hereditary elliptocytosis (HE) is *true?*
   A. Characteristic oval shape occurs in mature erythrocytes.
   B. Heterogeneous group of disorders linked to Rh-null individuals.
   C. Cellular defect involves the lipid composition of the membrane.
   D. HE cells are abnormally permeable to calcium.

111. Which of the following disorders is *not* commonly linked to the development of anemia of chronic disease?
   A. Persistent infections
   B. Noninfectious inflammatory disorders
   C. Chronic gastrointestinal blood loss
   D. Malignancy

112. Which of the following statements about hemoglobin C disease is *false?*
   A. Electrophoresis shows approximately 60% hemoglobin A and 40% hemoglobin C.
   B. Target cells are frequently seen on peripheral smears.
   C. Red cells may contain bar-shaped intracellular crystals.
   D. The disorder is less severe than sickle cell disease.

113. Which of the following is associated with sickle cells?
   A. Increased oxygen tension promotes sickling.
   B. There is decreased mechanical fragility.
   C. There is increased deformability.
   D. Increased sickling occludes vessels.

114. A bone marrow M:E ratio of 4:1 would be an expected finding for
   A. Sickle cell anemia
   B. Aplastic anemia
   C. Beta-thalassemia major
   D. Megaloblastic anemia

115. An elderly man with a 10-year history of chronic lymphocytic leukemia presented with jaundice and fatigue that was attributed to a recent 3-gram drop in his hemoglobin. Many spherocytes and polychromatophilic red cells were found on his Wright's stained blood smear. Which type of immune hemolytic anemia is most likely?
   A. Idiopathic warm autoimmune hemolytic anemia
   B. Secondary warm autoimmune hemolytic anemia
   C. Primary cold hemagglutinin disease
   D. Paroxysmal cold hemoglobinuria

116. A moderately anemic patient with suspected pernicious anemia (PA) shows intrinsic factor antibodies and a low cobalamin level. Which of the following would *not* support the diagnosis of PA?
   A. Gastric atrophy and achlorhydria
   B. Oval macrocytes and Howell-Jolly bodies
   C. Bone marrow erythroid precursors exhibit normoblastic maturation.
   D. Elevated serum lactate dehydrogenase (LD) and bilirubin levels

117. A cellulose acetate electrophoresis revealed a large band of hemoglobin in the hemoglobin S position. This band quantified at 95%. The peripheral smear revealed 70% target cells, and the solubility test was negative. Based on this information, what is the hemoglobin?
   A. Hemoglobin C
   B. Hemoglobin D
   C. Hemoglobin E
   D. Hemoglobin S

118. A previously healthy man experiences weakness and hemoglobinuria after taking the antimalarial agent primaquine. This hemolytic attack most likely occurred because of a deficiency of
   A. Pyruvate kinase
   B. Glucose-6-phosphate dehydrogenase
   C. 2,3-Bisphosphoglycerate
   D. Methemoglobin reductase

119. Which of the following is an acquired red cell membrane defect that results in increased sensitivity to complement binding?
A. March hemoglobinuria
B. Paroxysmal nocturnal hemoglobinuria
C. Paroxysmal cold hemoglobinuria
D. Methemoglobinemia

120. Which of the following is *not* associated with acquired reversible sideroblastic anemias?
A. Methotrexate therapy
B. Lead intoxication
C. Isoniazid treatment for tuberculosis
D. Acute alcohol ingestion

121. Which of the following statements about the relative anemia of pregnancy is *false?*
A. It is due to a reduction in the number of erythrocytes.
B. It is normocytic and normochromic.
C. It does not produce an oxygen deficit for the fetus.
D. It is associated with an increase in plasma volume.

122. The anemia found in chronic renal failure is most likely caused by
A. Loss of erythropoietin synthesis
B. Lack of cellular oxygen demand
C. Defective iron absorption
D. Destruction of red cells by uremic metabolites

123. Which of the following phrases about aplastic anemia is *false?*
A. Stem cell disorder
B. Risk of life-threatening infection
C. Frequent bleeding complications
D. Reduced red cell survival

124. The fish tapeworm *Diphyllobothrium latum* is associated with the development of
A. Microcytic anemia
B. Macrocytic anemia
C. Hemolytic anemia
D. Hypoproliferative anemia

125. An increase in erythropoietin is *not* a normal compensating mechanism in which of the following conditions?
A. Renal tumors
B. Heavy smoking
C. Cardiovascular disease
D. Pulmonary disease

126. Thalassemias are the result of a
A. Structural defect in the heme portion of hemoglobin
B. Quantitative defect in globin-chain synthesis
C. Qualitative defect in globin-chain structure
D. Change in hemoglobin solubility properties

127. Which of the following characterizes iron-deficiency anemia?
A. Decreased serum iron, decreased transferrin saturation, normal ferritin
B. Decreased serum transferrin, decreased transferrin saturation, decreased ferritin
C. Increased serum transferrin, decreased transferrin saturation, decreased ferritin
D. Increased serum transferrin, increased transferrin saturation, decreased serum iron

128. Clinical manifestations of a homozygous mutation involving the beta-globin gene will most likely appear
A. During embryonic development
B. In the neonate at birth
C. No later than 3 weeks after birth
D. By 6 months of age

129. The hemolysis associated with infection by malaria organisms is due to the
   A. Release of merozoites from erythrocytes
   B. Invasion of erythrocytes by merozoites
   C. Host's immunologic response to infected erythrocytes
   D. Toxins produced by the malarial organism

130. A clinical laboratory scientist received a 5 mL EDTA tube that contained 0.5 mL of anticoagulated blood. A smear was prepared and stained with Wright's stain. When examined microscopically, the majority of cells appeared to have many evenly distributed, blunt spicules on the surface. How should this cellular appearance be interpreted?
   A. An anemic condition requiring further testing
   B. Spur cells caused by using incorrect technique during slide preparation
   C. Artifact caused by a dirty spreader slide
   D. Crenated cells caused by incorrect blood to anticoagulant ratio

131. A failure to generate sufficient ATP is characteristic of red blood cells with
   A. Pyruvate kinase deficiency
   B. Glucose-6-phosphate dehydrogenase deficiency
   C. Lipoprotein deficiency
   D. Hexokinase deficiency

132. When iron use exceeds absorption, which of the following occurs *first?*
   A. Hemoglobin level decreases.
   B. Iron stores are depleted.
   C. Transferrin synthesis increases.
   D. Excretion of iron decreases.

133. The major mechanism responsible for the anemia of chronic disease is
   A. Impaired release of storage iron because of increased hepcidin levels
   B. Damaged bone marrow stem cells
   C. Immune destruction caused by red cell autoantibodies
   D. Increased erythropoietin response by committed red cell progenitor cells

134. Which of the following is *not* a characteristic of the idiopathic type of sideroblastic anemia?
   A. Refractory to treatment
   B. Blocks in heme synthesis are unknown
   C. Reversible with intramuscular vitamin B_{12} injections
   D. Subtype of myelodysplastic syndromes

135. Thinning of bones and deformation of facial bone structure seen in homozygous beta-thalassemia is a
   A. Consequence of disturbances in calcium metabolism
   B. Result of hyperplastic marrow activity
   C. Secondary disorder due to immunologic response
   D. Result of increased fibroclast activity

136. Which of the following does *not* accurately describe cold autoimmune hemolytic anemia?
   A. Red cell agglutination in extremities induces Raynaud's phenomenon.
   B. It may occur secondary to *Mycoplasma* pneumonia.
   C. Hemolysis is complement-mediated or via removal of coated cells.
   D. The autoantibody is usually an IgG type directed against Rh antigens.

137. Which of the following represents an anemia that would have a high red cell distribution width (RDW)?
A. Sickle cell disease during crisis
B. Thalassemia minor
C. Aplastic anemia
D. Anemia of chronic disorders

138. In which of the following disorders would splenomegaly *not* be a common finding?
A. Homozygous beta-thalassemia
B. Hereditary spherocytosis
C. Hemoglobin SC disease
D. Folic acid deficiency

Leukocytes

139. Functionally, white blood cells are divided into
A. Granulocytes, nongranulocytes
B. Polymorphonuclears, mononuclears
C. Phagocytes, immunocytes
D. Granulocytes, lymphocytes

140. What is the largest white blood cell normally found in the peripheral blood?
A. Eosinophil
B. Neutrophil
C. Lymphocyte
D. Monocyte

141. What is the approximate amount of time a granulocyte spends in the circulation before migrating into the tissues?
A. Less than 1 day
B. About 3 days
C. Up to 5 days
D. More than 10 days

142. What percentage of neutrophils in the peripheral blood constitutes the circulating pool?
A. 100%
B. 80%
C. 50%
D. 30%

143. What is the major phagocytic cell involved in the initial defense against bacterial pathogens?
A. Neutrophil
B. Eosinophil
C. Basophil
D. Monocyte

144. What is the growth factor that is primarily responsible for regulating granulocyte and monocyte production?
A. Erythropoietin
B. Colony stimulating factor
C. Interleukin
D. Thrombopoietin

145. What does the granulocyte mitotic pool in the bone marrow contain?
A. Myeloblasts and promyelocytes
B. Band and segmented forms
C. The majority of marrow granulocytes
D. Myelocytes and metamyelocytes

146. A "shift to the left," when used to describe a cell population, refers to
A. Increased cells in the blood due to a redistribution of blood pools
B. An increase in immature blood cells following release of bone marrow pools
C. A cell production "hiatus" or gap
D. A higher percentage of lymphocytes than neutrophils

147. Which of the following is characteristic of agranulocytosis?
A. Neutrophils without granules
B. Decreased numbers of granulocytes, red cells, and platelets
C. Immature granulocytes in the peripheral blood
D. Decreased numbers of granulocytes

148. Which of the following is *not* a characteristic of T lymphocytes?
   A. Secrete cytokines
   B. Synthesize antibody
   C. Comprise majority of cells in the blood lymphocyte pool
   D. Regulate the immune response

149. An adult has a total white blood cell count of 4.0×10^9/L ($4.0 \times 10^3/\mu$L). The differential count is as follows: polymorphonuclear neutrophils (PMNs) 25%, bands 5%, lymphocytes 65%, and monocytes 5%. The absolute value reference range for lymphocytes is $1.0–4.0 \times 10^9$/L. Which of the following is *true?*
   A. The percentage of lymphocytes is normal.
   B. There is an absolute lymphocytosis.
   C. There is a relative lymphocytosis.
   D. There is both an absolute and a relative lymphocytosis.

150. Which of the following statements is *correct?*
   A. Hypersegmented neutrophils have four nuclear lobes.
   B. Auer rods are composed of fused primary granules.
   C. Toxic granules are prominent secondary granules.
   D. Döhle bodies are agranular patches of DNA.

151. Which of the following factors is *not* associated with variations in the total white blood cell count?
   A. Age
   B. Exercise
   C. Emotional stress
   D. Sex

152. Of the following, an absolute neutrophil count of 1.0×10^9/L would be associated with
   A. Shortness of breath
   B. Bleeding tendencies
   C. Risk of infection
   D. No clinical symptoms

153. Which of the following statements about basophils is *false?*
   A. Morphologically, basophils resemble tissue mast cells.
   B. Membrane receptors bind IgG, initiating anaphylactic reactions.
   C. Basophilic granules contain heparin and histamine.
   D. Granules are water soluble.

154. The most mature granulocyte precursor that can undergo mitosis is the
   A. Myeloblast
   B. Promyelocyte
   C. Myelocyte
   D. Metamyelocyte

155. Production of primary granules ceases and production of secondary granules commences with what cell stage?
   A. Myeloblast
   B. Promyelocyte
   C. Myelocyte
   D. Metamyelocyte

156. Which of the following statements about eosinophils is *false?*
   A. They contain a type of peroxidase that is distinct from that of neutrophils.
   B. Eosinophilic granules contain lysozyme.
   C. Eosinophils are an important line of defense against parasites.
   D. Major basic protein is a component of eosinophil granules.

157. Which of the following is characteristic of primary granules?
   A. Coated with a phospholipid membrane
   B. Called azurophilic or specific granules
   C. Contain myeloperoxidase and lactoferrin
   D. Present in the promyelocyte stage only

158. Which of the following are indicators of a neutrophilic response to tissue damage or inflammatory stimuli?
   A. Toxic granules and Döhle bodies in the neutrophils
   B. Vacuoles and Barr bodies in the neutrophils
   C. Hypersegmented neutrophils and Auer rods
   D. Pyknotic neutrophils and Russell bodies

159. What is the term for cell movement through blood vessels to a tissue site?
   A. Diapedesis
   B. Opsonization
   C. Margination
   D. Chemotaxis

160. Vasodilation and bronchoconstriction are the result of degranulation by which of the following blood cells?
   A. Eosinophils
   B. Monocytes
   C. Neutrophils
   D. Basophils

161. On what basis can B and T lymphocytes be distinguished?
   A. Differences in nuclear shape
   B. Monoclonal antibody reactions to surface and cytoplasmic antigens
   C. Cytoplasmic granularity and overall cell size
   D. Chromatin pattern in the nucleus

162. Cells that produce immunoglobulins in response to antigenic stimulation are designated
   A. Natural killer cells
   B. Plasma cells
   C. Virocytes
   D. Thymocytes

163. Which of the following statements about neutrophils is *false?*
   A. Suppress allergic reactions caused by basophils
   B. Have surface receptors for IgG and complement components
   C. Contain alkaline phosphatase and muramidase
   D. Act in nonspecific phagocytosis and are destined to die

164. Which of the following characteristics would be *least* likely to distinguish reactive lymphocytes from monocytes?
   A. Sharp indentation of the cytoplasmic margin by adjacent red blood cells
   B. Presence of large azurophilic granules
   C. Irregular, indented nuclear shape
   D. Abundant, deeply basophilic cytoplasm

165. Which of the following can differentiate metamyelocytes from other stages of granulocyte maturation?
   A. Presence of specific granules
   B. Indentation of nucleus
   C. Absence of nucleoli
   D. Color of cytoplasm

166. Lymphocyte concentrations in the peripheral blood are greatest during what age interval?
   A. 1 to 4 years
   B. 4 to 15 years
   C. 16 to 40 years
   D. 40 to 70 years

167. Which of the following is the *least* likely to be expressed by early B cell precursors?
 A. SIgM, a surface membrane immunoglobulin
 B. CD34, a hematopoietic stem cell marker
 C. TdT (terminal deoxynucleotidyl transferase), a nuclear enzyme
 D. CD10 (CALLA), a surface antigen

168. Which of the following statements about macrophages is *incorrect?*
 A. They are mature tissue forms of blood monocytes.
 B. They serve as antigen-presenting cells to the immune system.
 C. Their quantity of lysosomes and acid hydrolases decreases during maturation.
 D. They remove damaged or dying cells and cellular debris.

169. Antigen-dependent lymphopoiesis occurs in secondary lymphoid tissue located in the
 A. Liver and kidney
 B. Spleen and lymph nodes
 C. Lungs and Peyer's patches
 D. Thymus and bone marrow

170. Which of the following is *not* produced by neutrophils during the respiratory burst?
 A. Hydroxyl radicals (OH^-)
 B. Hydrogen peroxide (H_2O_2)
 C. Superoxide anion (O_2^-)
 D. Myeloperoxidase

Leukocyte Disorders

171. In patients with infectious mononucleosis, which blood cells are infected by the causative agent?
 A. Monocytes
 B. T lymphocytes
 C. B lymphocytes
 D. Histiocytes

172. Which of the following statements about hairy cell leukemia is *true?*
 A. It is an acute disease, primarily affecting young adults.
 B. Splenomegaly is an unusual finding.
 C. Hairy cells contain tartrate-resistant acid phosphatase.
 D. Hairy cells are abnormal T lymphocytes.

173. Based on the WHO classification system, B cell ALL (FAB type L3) and ________________ represent different clinical presentations of the same disease entity.
 A. Burkitt lymphoma
 B. Hodgkin lymphoma
 C. Mycosis fungoides
 D. Small lymphocytic lymphoma

174. The presence of both immature neutrophils and nucleated erythrocytes in the peripheral blood is most accurately called a
 A. Neutrophilic left shift
 B. Regenerative left shift
 C. Neutrophilic leukemoid reaction
 D. Leukoerythroblastic reaction

175. In which anomaly is a failure of granulocytes to divide beyond the band or two-lobed stage observed?
 A. Pelger-Huët
 B. May-Hegglin
 C. Alder-Reilly
 D. Chédiak-Higashi

176. In which of the following are eosinophils *not* increased?
 A. Cushing syndrome
 B. Allergic disorders
 C. Skin disorders
 D. Parasitic infection

177. Which of the following represents the principal defect in chronic granulomatous disease (CGD)?
A. Chemotactic migration
B. Phagocytosis
C. Lysosomal formation and function
D. Oxidative respiratory burst

178. The blood shown in Color Plate 11■ is from a leukemia patient following treatment. These findings are most suggestive of therapy with
A. Corticosteroids (e.g., prednisone)
B. A folate antagonist (e.g., methotrexate)
C. Recombinant erythropoietin
D. Chloramphenicol

179. A patient with normal hemoglobin and WBC count values, a persistently elevated platelet count (over 1000×10^9/L), increased marrow megakaryocytes, and a history of frequent bleeding and clotting episodes most likely has
A. Polycythemia vera
B. Chronic myelofibrosis
C. Essential thrombocythemia
D. Chronic myelogenous leukemia

180. An adult patient with massive splenomegaly has mild anemia, a slightly elevated WBC count, and an LAP score of 170. The blood smear shows teardrop erythrocytes and leukoerythroblastosis. These findings are most consistent with
A. Chronic myelogenous leukemia
B. Idiopathic myelofibrosis
C. Primary polycythemia
D. Primary thrombocythemia

181. Which of the following infections does *not* reveal a blood picture as seen in Color Plate 12■?
A. Epstein-Barr virus (EBV)
B. *Bordetella pertussis* (whooping cough)
C. Cytomegalovirus (CMV)
D. *Toxoplasma gondii* (toxoplasmosis)

182. The most common type of chronic lymphocytic leukemia (CLL) in the United States involves the
A. B cell
B. NK cell
C. T cell
D. Plasma cell

183. Which of the following are characteristic findings in Waldenström disease?
A. Increased IgA and hepatosplenomegaly
B. Increased IgE and renal failure
C. Increased IgG and hypercalcemia
D. Increased IgM and blood hyperviscosity

184. Which of the following would *not* cause a total WBC count of 62.2×10^9/L ($62.2 \times 10^3/\mu$L) and the blood findings seen in Color Plate 13■?
A. Treatment with myeloid growth factors
B. Gram-negative septicemia
C. Human immunodeficiency virus (HIV)
D. Systemic fungal infection

185. The peripheral blood shown in Color Plate 14■ is from a 69-year-old female. Her WBC count was 83.0×10^9 cells/L ($83.0 \times 10^3/\mu$L) and her platelet count was normal. Based on the cell morphology and this information, what is the most likely diagnosis?
A. Acute lymphoblastic leukemia
B. Chronic lymphocytic leukemia
C. Waldenström macroglobulinemia
D. Viral infection

186. In which of the following is progression to acute leukemia *least* likely?
A. Chronic myelogenous leukemia (CML)
B. Refractory anemia with excess blasts (RAEB)
C. Refractory anemia with ringed sideroblasts (RARS)
D. Chronic lymphocytic leukemia (CLL)

187. A Gaucher cell is best described as a macrophage with
   A. "Wrinkled" cytoplasm due to an accumulation of glucocerebroside
   B. "Foamy" cytoplasm filled with unmetabolized sphingomyelin
   C. Pronounced vacuolization and deposits of cholesterol
   D. Abundant cytoplasm containing storage iron and cellular remnants

188. Which of the following suggests a diagnosis of Hodgkin disease rather than other lymphoproliferative disorders?
   A. Presence of a monoclonal population of large lymphoid cells
   B. Predominance of immature B cells with irregular nuclear clefts
   C. Circulating T cells with a convoluted, cerebriform nucleus
   D. Presence of giant binucleated Reed-Sternberg cells with prominent nucleoli

189. In a patient with fever of unknown origin, which of the following findings is *not* consistent with an inflammatory process?
   A. Increased C-reactive protein
   B. Increased albumin level
   C. Increased fibrinogen level
   D. Increased erythrocyte sedimentation rate

190. The presence of the chromosomal abnormality t(15;17) and a high incidence of disseminated intravascular coagulation (DIC) is diagnostic of
   A. Acute myeloblastic leukemia without maturation (FAB type M1)
   B. Acute myeloblastic leukemia with maturation (FAB type M2)
   C. Acute promyelocytic leukemia (FAB type M3)
   D. Acute myelomonocytic leukemia (FAB type M4)

191. Which of the following is *not* commonly found in acute myelogenous leukemias?
   A. Neutropenia
   B. Thrombocytopenia
   C. Hepatosplenomegaly
   D. Lymphadenopathy

192. The child whose blast cells are shown in Color Plate 15■ has acute lymphoblastic leukemia that is precursor B cell type and CALLA positive. Analysis by flow cytometry would likely show cells that immunophenotype for
   A. CD2, CD7
   B. CD10, CD19
   C. CD13, CD33
   D. CD14, CD34

193. The patient whose bone marrow is shown in Color Plate 16■ most likely has a(n)
   A. Acute leukemia
   B. Chronic leukemia
   C. Myelodysplastic syndrome
   D. Aplastic anemia

194. Multiple myeloma is characterized by the presence in urine of large amounts of
   A. Cryoglobulins
   B. IgG heavy chains
   C. IgG light chains
   D. Beta microglobulins

195. Which of the following is *not* classified as a myeloproliferative disorder?
   A. Polycythemia vera
   B. Essential thrombocythemia
   C. Multiple myeloma
   D. Chronic myelogenous leukemia

196. Which of the following gene mutations correlates with the t(9;22) that is present in Philadelphia chromosome positive chronic myelogenous leukemia?
   A. MYC/IGH
   B. BCR/ABL
   C. PML/RARA
   D. JAK2

197. Which of the following statements does *not* correctly describe the WHO (World Health Organization) classification of hematopoietic neoplasms?
   A. Acute leukemia is defined as the presence of at least 20% bone marrow blasts.
   B. Diagnosis is based on cellular morphology and cytochemistry.
   C. It groups lymphoid disorders into B cell, T/NK cell, and Hodgkin lymphoma.
   D. Diagnostic criteria include morphologic, cytochemical, immunologic, cytogenetic, and molecular features.

198. Which of the following would be *least* helpful in distinguishing chronic myelogenous leukemia (CML) from a neutrophilic leukemoid reaction?
   A. An extreme leukocytosis with increased neutrophilic bands, metamyelocytes, and myelocytes
   B. Leukocyte alkaline phosphatase score
   C. Presence of marked splenomegaly
   D. Neutrophils with Döhle bodies and toxic granulation

199. The cytoplasmic inclusion present in the cell shown in Color Plate 17■
   A. Excludes a diagnosis of acute myelogenous leukemia
   B. Stains positive with leukocyte alkaline phosphatase (LAP)
   C. Stains positive with myeloperoxidase (MPO)
   D. Identifies the cell as a malignant lymphoblast

200. Which of the following is a typical finding in chronic leukemias at onset?
   A. Symptoms of infection and bleeding
   B. Significant thrombocytopenia
   C. Severe anemia
   D. Elevated leukocyte count

201. In what condition would an LAP score of 10 most likely be found?
   A. Bacterial septicemia
   B. Late pregnancy
   C. Polycythemia vera
   D. Chronic myelogenous leukemia

202. Which of the following is *not* associated with neutrophilia?
   A. Staphylococcal pneumonia
   B. Crushing injury
   C. Infectious hepatitis
   D. Neoplasms (tumors)

203. In which of the following would an absolute monocytosis *not* be seen?
   A. Tuberculosis
   B. Recovery stage of acute bacterial infection
   C. Collagen disorders
   D. Infectious mononucleosis

204. Coarse PAS positivity may be found in the leukemic cells of
   A. Acute myeloblastic leukemia (FAB type M1)
   B. Acute lymphoblastic leukemia (FAB type L1)
   C. Acute myelomonocytic leukemia (FAB type M4)
   D. Acute monocytic leukemia (FAB type M5)

205. Which of the following is *not* among the diagnostic criteria used for classifying the myelodysplastic syndromes?
   A. Unexplained anemia refractory to treatment
   B. Hypogranular and hyposegmented neutrophils
   C. Abnormal platelet size and granulation
   D. Hypocellular bone marrow with 25% blasts

206. Naphthol AS-D chloroacetate esterase (specific) is usually positive in _______________ cells, and alpha-naphthyl acetate esterase (nonspecific) is useful for identifying blast cells of _______________ lineage.
   A. Granulocytic; monocytic
   B. Monocytic; granulocytic
   C. Granulocytic; lymphocytic
   D. Monocytic; lymphocytic

207. The familial disorder featuring pseudo-Döhle bodies, thrombocytopenia, and large platelets is called
   A. May-Hegglin anomaly
   B. Chédiak-Higashi syndrome
   C. Pelger-Huët anomaly
   D. Alder-Reilly anomaly

208. Alder-Reilly anomaly is an abnormality of
   A. Lysosomal fusion
   B. Nuclear maturation
   C. Oxidative metabolism
   D. Mucopolysaccharide metabolism

209. What is the initial laboratory technique for the diagnosis of monoclonal gammopathies?
   A. Immunologic markers of marrow biopsy cells
   B. Cytochemical staining of marrow and peripheral blood cells
   C. Serum and urine protein electrophoresis
   D. Cytogenetic analysis of marrow cells

210. Which of the following statements about Hodgkin disease is *false?*
   A. Peak incidence occurs in young adults.
   B. Staging determines extent of disease and treatment course.
   C. Stage IV has the best prognosis.
   D. Almost a 2:1 male predominance over females is characteristic.

211. The blast cells shown in Color Plate 18■ are CD14 and CD33 positive, Sudan black B positive, specific esterase positive, and nonspecific esterase positive. Which type of acute leukemia is most consistent with the immunophenotyping and cytochemical staining results?
   A. Acute lymphoblastic leukemia, T cell type
   B. Acute erythroleukemia
   C. Acute myelomonocytic leukemia
   D. Acute monocytic leukemia

212. Which type of leukemia is associated with the best prognosis for a cure?
   A. Chronic lymphocytic leukemia in the elderly
   B. Acute lymphoblastic leukemia in children
   C. Acute myelogenous leukemia in children
   D. Chronic myelogenous leukemia in young adults

213. What is the key diagnostic test for Hodgkin lymphoma?
   A. Bone marrow biopsy
   B. Lymph node biopsy
   C. Spinal tap
   D. Skin biopsy

214. A bone marrow with 90% cellularity and myeloid:erythroid (M:E) ratio of 10:1 is most characteristic of
   A. Chronic myelogenous leukemia
   B. Primary polycythemia
   C. Beta-thalassemia major
   D. Aplastic anemia

215. A 60-year-old patient presents with extreme fatigue. Her blood and bone marrow findings are as follows: severe anemia with a dual RBC population, 3% marrow blasts, and numerous ringed sideroblasts. This information is most consistent with
   A. Refractory anemia (RA)
   B. Refractory anemia with ringed sideroblasts (RARS)
   C. Refractory anemia with excess blasts (RAEB)
   D. Chronic myelomonocytic leukemia (CMML)

216. Which of the following is *not* a mechanism by which neutropenia may be produced?
   A. Hypersplenism
   B. Marrow injury or replacement
   C. Recent strenuous exercise
   D. Drug-induced antibodies

217. Which of the following is *not* a characteristic finding in polycythemia vera?
   A. Blood pancytosis
   B. Increased red cell mass
   C. Increased erythropoietin level
   D. Increased blood viscosity

218. In what disorder is significant basophilia most commonly seen?
   A. Hairy cell leukemia
   B. Plasma cell leukemia
   C. Acute lymphoblastic leukemia
   D. Chronic myelogenous leukemia

219. Acute erythroleukemia (FAB type M6) is characterized by increased
   A. Promyelocytes and lysozyme activity
   B. Marrow megakaryocytes and thrombocytosis
   C. Marrow erythroblasts and multinucleated red cells
   D. Marrow monoblasts and immature monocytes

220. The blood findings present in Color Plate 20■ are from a patient with complaints of fatigue and severe lower back pain. Which of the following would *not* be typical of this disease?
   A. Bone tumors of plasma cells
   B. Hypercalcemia
   C. Progressive renal impairment
   D. Normal sedimentation rate

221. Myeloid metaplasia refers to
   A. Displacement of normal marrow cells by fibrous tissue
   B. Hematopoietic failure
   C. Extramedullary hematopoiesis
   D. Tumors (neoplasms) of the bone marrow

222. Which of the following statements about non-Hodgkin types of lymphoma is *true?*
   A. Lymphadenopathy is the most common presenting symptom.
   B. Initially, they present as a systemic disease rather than a localized tumor.
   C. They are often associated with multiple bone lesions.
   D. They are characterized by proliferation of malignant cells primarily involving the bone marrow.

Methodology

223. What combination of reagents is used to measure hemoglobin?
   A. Hydrochloric acid and *p*-dimethylaminobenzaldehyde
   B. Potassium ferricyanide and potassium cyanide
   C. Sodium bisulfite and sodium metabisulfite
   D. Sodium citrate and hydrogen peroxide

224. The slowest-moving hemoglobin(s) on an alkaline electrophoresis at pH 8.6 is(are)
   A. A
   B. A_2, C, E, and O
   C. F
   D. S, D, and G

225. A patient with suspected sickle cell trait has negative solubility test results, but hemoglobin electrophoresis at pH 8.6 shows an apparent A-S pattern. What is the most likely explanation?
   A. Patient has hemoglobin AS, and the solubility test is incorrect.
   B. Patient has hemoglobin AA, and the electrophoresis is incorrect.
   C. Patient has hemoglobin AD or AG, and both procedures are correct.
   D. Tests need to be repeated; impossible to determine which procedure is correct.

226. Which of the following is an *incorrect* statement about the solubility test for Hemoglobin S?
   A. Hemoglobin S polymerizes when deoxygenated.
   B. Testing performed on a 2-day-old infant can result in a false negative result.
   C. Sickle cell trait can be differentiated from sickle cell anemia with this test.
   D. The test is positive in hemoglobin C_{Harlem}.

227. Which of the following is *not* associated with causing a falsely low ESR?
   A. Column used is slanted.
   B. EDTA tube is clotted.
   C. EDTA tube is one-third full.
   D. EDTA specimen is 24 hours old.

228. A platelet count is performed on an automated instrument from an EDTA blood sample. Smear evaluation reveals the presence of platelet clumps. The specimen is redrawn using sodium citrate as the anticoagulant, and a count of 300×10^9/L is obtained. What is the correct platelet count to report?
   A. 270×10^9/L
   B. 300×10^9/L
   C. 330×10^9/L
   D. 360×10^9/L

229. To best preserve cellular morphology, differential smears from an EDTA specimen should be made no more than ______________ hour(s) after collection.
   A. 1
   B. 5
   C. 12
   D. 24

230. The blood smear made on a patient with polycythemia vera is too short. What should be done to correct this problem?
   A. Decrease the angle of the spreader slide.
   B. Increase the angle of the spreader slide.
   C. Adjust the angle of the spreader slide to 45 degrees.
   D. Use a smaller drop of blood.

231. The components of Wright's stain include
   A. Crystal violet and safranin
   B. Brilliant green and neutral red
   C. New methylene blue and carbolfuchsin
   D. Methylene blue and eosin

232. What is the reason for red blood cells to be bright red and the WBC nuclei to be poorly stained when using Wright's stain?
   A. The staining time is too long.
   B. The stain or buffer is too alkaline.
   C. The stain or buffer is too acidic.
   D. The smear was not washed long enough.

233. If 60 reticulocytes are counted in 1000 red blood cells, what is the reticulocyte count?
   A. 0.06%
   B. 0.6%
   C. 6.0%
   D. 60.0%

234. Using the percent reticulocyte from question 233 and an RBC count of 3.00×10^{12}/L ($3.00 \times 10^6/\mu$L), the calculated absolute reticulocyte count reported in SI units is
   A. 1.8×10^9/L
   B. 18×10^9/L
   C. 180×10^9/L
   D. $180 \times 10^3/\mu$L

235. The Sudan black B stain shown in Color Plate 19■ is a stain for
A. Glycogen
B. Lipids
C. Myeloperoxidase
D. Acid phosphatase

236. The following numbers were obtained in evaluating leukocyte alkaline phosphatase (LAP) activity in neutrophils. What is the score?

0	1	2	3	4
15	20	30	20	15

A. 100
B. 115
C. 200
D. 215

237. Perl's Prussian blue is a stain used to detect
A. DNA
B. RNA
C. Iron
D. Glycogen

238. Which of the following red cell inclusions stain with *both* Perl's Prussian blue and Wright's stain?
A. Howell-Jolly bodies
B. Basophilic stippling
C. Pappenheimer bodies
D. Heinz bodies

239. What is the depth between the counting platform and the coverslip on a hemacytometer?
A. 0.01 mm
B. 0.10 mm
C. 1.00 mm
D. 0.1 cm

240. A WBC count is performed on a hemacytometer using a 1:20 dilution. 308 cells are seen in a total area of 8 mm^2. What is the WBC count?
A. 3.8×10^9/L
B. 7.7×10^9/L
C. 15.4×10^9/L
D. 38.5×10^9/L

241. Which set of results indicates that an error in measurement has occurred?

	RBC $\times 10^{12}$/L	Hgb (g/dL)	Hct (%)
A.	2.50	7.6	22.9
B.	2.75	9.5	24.8
C.	3.40	10.0	31.0
D.	3.75	11.1	34.0

242. Which of the following would *not* be the cause of a falsely high MCHC of 38.3 g/dL on an automated instrument?
A. Hereditary spherocytosis
B. Lipemia
C. Presence of a cold agglutinin
D. Instrument sampling or mixing error

243. What is the principle of automated impedance cell counters?
A. Angle of laser beam scatter by cells
B. Amplification of an electrical current by cells
C. Interruption of an electrical current by cells
D. Change in optical density of the solution containing cells

244. A clinically significant difference between two electronic cell counts is indicated when the standard deviation is greater than
A. ±1.0
B. ±1.5
C. ±2.0
D. ±3.0

245. Side angle scatter in a laser-based cell counting system is used to measure
A. Cell size
B. Cytoplasmic granularity
C. Cell number
D. Immunologic (antigenic) identification

246. A white blood cell count is done on an automated impedance cell counter from a patient with the blood picture seen in Color Plate 4■. The WBC count is most likely
A. Falsely increased because of nRBCs
B. Falsely increased because of red cell fragments
C. Falsely decreased because of nRBCs
D. Accurate; no error with this methodology

247. The hemoglobin A_2 quantification using anion exchange chromatography will be valid in
A. Hemoglobin C disease
B. Hemoglobin E trait
C. Hemoglobin O trait
D. Beta-thalassemia minor

248. Which of the following is *not* associated with an increased osmotic fragility and a decreased surface area-to-volume ratio?
A. Beta-thalassemia major
B. Hereditary spherocytosis
C. Warm autoimmune hemolytic anemia
D. Burn victims

249. A clotted EDTA tube can be used to perform a(n)
A. Erythrocyte sedimentation rate
B. Solubility test for hemoglobin S
C. Hematocrit
D. Platelet count

250. The test value range that includes 95% of the normal population is the
A. Reference interval
B. Linearity limit
C. Reportable range
D. Critical range

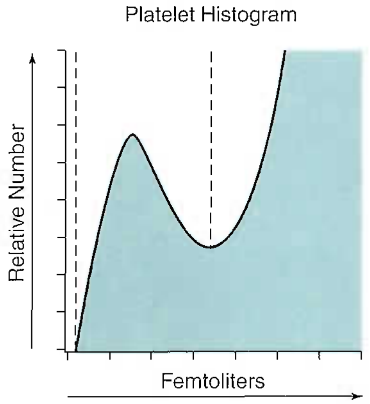
251. To establish a standard curve for reading hemoglobin concentration,
A. A commercial control material is used.
B. A wavelength of 640 nm is employed.
C. Certified standards are used.
D. A patient blood sample of known hemoglobin concentration is used.

252. Which of the following is *not* a source of error when measuring hemoglobin by the cyanmethemoglobin method?
A. Excessive anticoagulant
B. White blood cell count that exceeds linearity limits
C. Lipemic plasma
D. Scratched or dirty hemoglobin measuring cell

253. Which of the following statements about microhematocrits is *false?*
A. Excessive centrifugation causes falsely low results.
B. A tube less than half full causes falsely low results.
C. Hemolysis causes falsely low results.
D. Trapped plasma causes falsely high results.

254. The erythrocyte sedimentation rate (ESR) is influenced by the red cell phenomenon seen in Color Plate 20■. Which of the following factors will neither contribute to this phenomenon nor affect the ESR?
A. Size of the red blood cells
B. Shape of the red blood cells
C. Hemoglobin content of the red blood cells
D. Composition of the plasma

255. An EDTA blood sample run on an automated impedance cell counter has generated a warning flag at the upper region of the platelet histogram illustrated below. Which of the following would *not* be a cause of this warning flag?
   A. Nucleated RBCs
   B. Microcytic RBCs
   C. EDTA-dependent platelet agglutinins
   D. Giant platelets



256. To evaluate normal platelet numbers in an appropriate area of a blood smear, approximately how many platelets should be observed per oil immersion field?
   A. 1–4
   B. 4–10
   C. 8–20
   D. 20–50

257. Which of the following statements about manual reticulocyte counts is *false?*
   A. The blood/stain mixture is incubated for 5–10 minutes.
   B. New methylene blue, a supravital stain, is used.
   C. RBC inclusions can result in falsely elevated counts.
   D. An erythrocyte must have at least 4 blue particles to be counted as a reticulocyte.

258. When are automated cell counters required to have a calibration check performed?
   A. At least every 3 months
   B. After replacement of any major part
   C. After performing monthly maintenance
   D. When the control values are greater than 2 standard deviations from the mean

259. A blood sample was run through an automated cell counter and the following results were obtained: WBC 6.9×10^9/L ($6.9 \times 10^3/\mu$L), RBC 3.52×10^{12}/L ($3.52 \times 10^6/\mu$L), Hgb 120 g/L (12.0 g/dL), Hct 0.32 L/L (32.0%), MCH 34.1 pg, MCHC 37.5 g/dL. Which of the troubleshooting steps that follows should be performed to obtain reportable results?
   A. Perform a saline replacement procedure.
   B. Warm the specimen to 37°C and rerun.
   C. Perform a microhematocrit.
   D. None; the results are reportable.

260. Which of the following tests could be performed on a hemolyzed blood sample?
   A. Hemoglobin only
   B. Hemoglobin and platelet count
   C. RBC count and hematocrit
   D. No results would be reportable.

261. For which of the following procedures would heparin be a recommended anticoagulant?
   A. Platelet count
   B. Coagulation tests
   C. Smear-based red cell morphology
   D. Osmotic fragility

262. In the platelet count procedure using phase microscopy,
   A. Platelets appear dark against a light background.
   B. The entire ruled counting surface of the hemacytometer is used.
   C. Ammonium oxalate will lyse the WBCs.
   D. Platelets should be counted immediately after plating the hemacytometer.

263. What is the quality control term used to describe the reproducibility of a test?
   A. Accuracy
   B. Precision
   C. Standard deviation
   D. Specificity

Case Histories

Use the following information to answer questions 264–268.

The peripheral blood shown in Color Plate 4■ is from a 10-month-old Greek boy with the following results on an automated impedance counter: WBC 35.0×10^9/L ($35.0 \times 10^3/\mu$L); RBC 2.50×10^{12}/L ($2.50 \times 10^6/\mu$L); hemoglobin 45 g/L (4.5 g/dL); hematocrit 0.16 L/L (16%); platelet count 250×10^9/L (250,000/μL); reticulocyte count 8.0%; 110 nucleated red blood cells/100 WBCs and many targets are seen. Other laboratory results are as follows: serum iron elevated; total iron-binding capacity (TIBC) decreased; serum ferritin elevated.

264. What is the corrected white blood cell count expressed in SI units of $\times 10^9$/L?
   A. 4.6
   B. 12.5
   C. 16.7
   D. 18.4

265. What would be the appearance of the child's red blood cells on a peripheral smear?
   A. Microcytic, hypochromic
   B. Normocytic, hypochromic
   C. Normocytic, normochromic
   D. Microcytic, normochromic

266. The CBC, serum iron, total iron-binding capacity, and serum ferritin levels are most characteristic of
   A. Beta-thalassemia minor
   B. Iron-deficiency anemia
   C. Alpha-thalassemia minor
   D. Beta-thalassemia major

267. What type(s) of hemoglobin will be detected on this child using hemoglobin electrophoresis?
   A. A only
   B. A and F
   C. A, increased A_2, F
   D. F only

268. Why is it difficult to diagnose this disorder in a newborn?
   A. The liver is immature.
   B. The beta chains are not fully developed at birth.
   C. It is similar to hemolytic disease of the newborn (HDN) because of ABO incompatibility.
   D. There are normally many erythrocyte precursors in the peripheral blood.

Use the following information to answer questions 269–271.

A 75-year-old man with rheumatoid arthritis complains to his physician of pain and fatigue. His CBC results are as follows: WBC 6.8×10^9/L ($6.8 \times 10^3/\mu L$); RBC 3.49×10^{12}/L ($3.49 \times 10^6/\mu L$); hemoglobin 97 g/L (9.7 g/dL); hematocrit 0.29 L/L (29%); MCV 83 fL; MCHC 33.9 g/dL. Other laboratory results are as follows: serum iron and total iron-binding capacity (TIBC) both decreased, serum ferritin slightly elevated.

269. If the serum iron is 22 μg/dL and the TIBC is 150 μg/dL, what is the percent transferrin?
   A. 7%
   B. 10%
   C. 12%
   D. 15%

270. The results of the CBC and iron studies in this case are most characteristic of
   A. Beta-thalassemia minor
   B. Iron deficiency
   C. Sideroblastic anemia
   D. Anemia of chronic disease

271. Which of the following is *not* associated with the anemia described in question 270?
   A. Chronic gastrointestinal blood loss
   B. Hodgkin lymphoma
   C. Tuberculosis
   D. Systemic lupus erythematosus

Use the following information to answer questions 272–274.

The peripheral blood shown in Color Plate 11 ■ is from a 19-year-old female college student who has been living primarily on tea, beer, and cereal for the past 9 months because she finds dining hall food distasteful. She visits student health complaining of fatigue. Her CBC results are as follows: WBC 2.5×10^9/L ($2.5 \times 10^3/\mu L$); RBC 2.10×10^{12}/L ($2.10 \times 10^6/\mu L$); hemoglobin 85 g/L (8.5 g/dL); hematocrit 0.24 L/L (24%); platelet count 110×10^9/L (110,000/μL); MCV 114 fL; MCHC 35.0 g/dL; reticulocyte count 0.8%.

272. What test(s) should be done *first* to determine a diagnosis in this patient?
   A. Vitamin B_{12} and folate levels
   B. Iron studies
   C. Bone marrow examination
   D. Osmotic fragility

273. In the absence of neurological symptoms, the anemia in this patient is most likely caused by a lack of
   A. An enzyme
   B. Iron
   C. Folic acid
   D. Intrinsic factor

274. Which of the following is *not* a laboratory finding in this general classification of anemia?
   A. Target cells and schistocytes
   B. Teardrop cells and macro-ovalocytes
   C. Howell-Jolly bodies and Cabot rings
   D. Elevated serum LD and iron levels

Use the following information to answer questions 275–277.

A 45-year-old Scandinavian woman with white hair appears older than her age. She complains to her physician of weakness, a tingling sensation in her lower extremities, and shortness of breath. Her CBC results are as follows: WBC 3.4×10^9/L ($3.4 \times 10^3/\mu$L); RBC 1.90×10^{12}/L ($1.90 \times 10^6/\mu$L); hemoglobin level 86 g/L (8.6 g/dL); hematocrit 0.25 L/L (25%); MCV 132 fL; MCHC 34.4 g/dL; platelet count 100×10^9/L (100,000/μL). Cabot rings are noted on the peripheral smear.

275. The clinical and laboratory findings are most consistent with
   A. Liver disease
   B. Pernicious anemia
   C. Folic acid deficiency
   D. Aplastic anemia

276. Which of the following is *not* associated with this disorder?
   A. Alcoholism
   B. Antibodies to intrinsic factor or parietal cells
   C. *Diphyllobothrium latum* infection
   D. Achlorhydria

277. Which of the following statements about megaloblastic anemia is *true?*
   A. Oral folate therapy reverses the neurologic symptoms of PA.
   B. Intramuscular injections of vitamin B_{12} will reverse the neurologic symptoms of PA.
   C. Methotrexate (chemotherapeutic agent) is a vitamin B_{12} antagonist.
   D. Folate deficiency takes years to develop.

Use the following information to answer questions 278–280.

A 32-year-old African-American traveling to Africa on business had been healthy until he began taking primaquine for prevention of malaria. He went to his physician because he felt faint and his urine was black. His CBC results are as follows: WBC 6.5×10^9/L ($6.5 \times 10^3/\mu$L); RBC 1.67×10^{12}/L ($1.67 \times 10^6/\mu$L); hemoglobin level 50 g/L (5.0 g/dL); hematocrit 0.15 L/L (15%); MCV 89.8 fL; MCHC 33.3 g/dL; platelet count 175×10^9/L (175,000/μL); reticulocyte 25.0%.

278. The most likely cause of this hemolytic episode is
   A. G6PD deficiency
   B. Hereditary spherocytosis
   C. Sickle cell disease
   D. Pyruvate kinase deficiency

279. The defect in this disorder is caused by an
   A. Amino acid substitution
   B. Intrinsic red blood cell membrane defect
   C. Enzyme deficiency in the hexose monophosphate shunt
   D. Enzyme deficiency in the Embden-Meyerhof pathway

280. Inclusions that form when the patient is oxidatively challenged are composed of
   A. RNA
   B. Denatured hemoglobin
   C. DNA
   D. Iron

Use the following information to answer questions 281–283.

A 15-month-old malnourished child is brought to the clinic for a routine examination. Her CBC results are as follows: WBC $9.5 \times 10^9/L$ ($9.5 \times 10^3/\mu L$); RBC $2.70 \times 10^{12}/L$ ($2.70 \times 10^6/\mu L$); hemoglobin 67 g/L (6.7 g/dL); hematocrit 0.25 L/L (25%); MCV 73.5 fL; MCHC 26.8 g/dL; reticulocyte 0.2%; RDW 19%. Abnormal RBC morphology present included pencil forms and target cells.

281. What is this toddler's most probable diagnosis?
   A. Folic acid deficiency
   B. Hereditary spherocytosis
   C. Iron deficiency
   D. Erythroblastosis fetalis

282. The earliest indicator of this disease state is
   A. Decreased folic acid
   B. Decreased serum iron
   C. Decreased serum ferritin
   D. Increased bilirubin

283. What is the toddler's absolute reticulocyte count?
   A. $0.05 \times 10^9/L$
   B. $0.5 \times 10^9/L$
   C. $5 \times 10^9/L$
   D. $50 \times 10^9/L$

Use the following information to answer questions 284–288.

An 8-year-old girl is seen by the family physician. On physical examination, the physician notes fever, sore throat, bruising, petechiae, and pallor. A CBC is drawn and the results are as follows: WBC $110 \times 10^9/L$ ($110 \times 10^3/\mu L$); RBC $1.70 \times 10^{12}/L$ ($1.70 \times 10^6/\mu L$); hemoglobin 55 g/L (5.5 g/dL); hematocrit 0.16 L/L (16%); differential count shows 93% blasts and 7% lymphocytes. A bone marrow examination is performed and reveals 85% blasts. All of the blasts are small with no variation in their appearance.

284. Which of the following would you expect to most accurately reflect the child's platelet count?
   A. $10 \times 10^9/L$
   B. $100 \times 10^9/L$
   C. $200 \times 10^9/L$
   D. $400 \times 10^9/L$

285. What is this child's most probable diagnosis?
   A. Acute lymphoblastic leukemia
   B. Acute myelogenous leukemia
   C. Hairy cell leukemia
   D. Myelodysplastic syndrome

286. Which of the following cytochemical stains would most likely be positive in the blast cells of this patient?
   A. Myeloperoxidase
   B. Leukocyte alkaline phosphatase
   C. Periodic acid–Schiff
   D. Nonspecific esterase

287. Terminal deoxyribonucleotidyl transferase (TdT) is present in
   A. Precursor B and precursor T lymphoid cells
   B. Mature B and T lymphocytes
   C. Precursor B cells and mature B lymphocytes
   D. Precursor T cells and mature T lymphocytes

288. The presence of CD2, CD5, CD7 and the *absence* of CD10 (CALLA) are associated with
   A. B lymphocytes
   B. T lymphocytes
   C. Myeloid cells
   D. Monocytic cells

Use the following information to answer questions 289–292.

The peripheral blood smear in Color Plate 17 ■ and the Sudan black B stain in Color Plate 19 ■ are from a 90-year-old man complaining of fatigue and nosebleeds. The physician noted the patient was febrile and had petechiae. CBC results were as follows: WBC 20.0×10^9/L ($20.0 \times 10^3/\mu$L); RBC 2.58×10^{12}/L ($2.58 \times 10^6/\mu$L); hemoglobin 77 g/L (7.7 g/dL); hematocrit 0.24 L/L (24%); platelet count 32×10^9/L (32,000/μL); differential count shows 75% blasts, 20% lymphocytes, and 5% segmented neutrophils. A bone marrow examination revealed 80% cellularity with 80% blasts. The blasts were myeloperoxidase and specific esterase positive; nonspecific esterase and PAS negative.

289. What is this patient's most likely diagnosis?
   A. Acute myelogenous leukemia without maturation (FAB type M1)
   B. Acute myelogenous leukemia with maturation (FAB type M2)
   C. Acute monocytic leukemia (FAB type M5)
   D. Myelodysplastic syndrome

290. Cytogenetic studies would most likely show which of the following chromosome abnormalities?
   A. t(8;21)
   B. t(8;14)
   C. t(9;22)
   D. t(15;17)

291. Using World Health Organization (WHO) criteria for the diagnosis of acute leukemia, the percentage of bone marrow blasts must be at least
   A. 5
   B. 20
   C. 30
   D. 50

292. Which of the following is *not* considered an underlying condition that predisposes a patient to acute leukemia?
   A. Viral infections
   B. Bacterial infections
   C. Chronic bone marrow dysfunction
   D. Congenital chromosome abnormalities

Use the following information to answer questions 293–296.

An 83-year-old woman is seen in the emergency department complaining of fatigue and recent weight loss. Her CBC results are as follows: WBC 2.6×10^9/L ($2.6 \times 10^3/\mu$L); RBC 2.79×10^{12}/L ($2.79 \times 10^6/\mu$L); hemoglobin 92 g/L (9.2 g/dL); hematocrit 0.28 L/L (28%); MCV 100.0 fL; RDW 23.5%; platelet count 42×10^9/L (42,000/μL); differential count shows 42% segmented neutrophils, 45% band neutrophils, 3% lymphocytes, 3% metamyelocytes, 4% myelocytes, 3% blasts, and 4 nRBC/100 WBC. Morphologic changes noted on the differential smear include poor granulation and hyposegmentation of the neutrophils, giant platelets that display poor granulation, oval macrocytes, basophilic stippling, Cabot rings, Pappenheimer bodies, and Howell-Jolly bodies. Three micromegakaryocytes are seen per 100 WBCs. Serum B_{12} and folate levels are normal.

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293. The peripheral blood findings are most consistent with
   A. Myelodysplastic syndrome
   B. Degenerative left shift
   C. Megaloblastic anemia
   D. Chronic myelogenous leukemia

294. The expected bone marrow findings in this disorder using WHO criteria are
   A. Hypocellular; blasts $\geq$20%
   B. Hypocellular; blasts <20%
   C. Hypercellular; blasts $\geq$20%
   D. Hypercellular; blasts <20%

295. If the bone marrow in this patient had 18% blasts, the most likely disorder would be
   A. Chronic myelomonocytic leukemia (CMML)
   B. Chronic myelogenous leukemia (CML)
   C. Refractory anemia with ringed sideroblasts (RARS)
   D. Refractory anemia with excess blasts (RAEB)

296. Which of the following is a *false* statement about myelodysplastic syndromes?
   A. MDS is "preleukemic" and frequently terminates in acute leukemia.
   B. Treatment for MDS is only supportive and not a cure.
   C. Median survival for all types of MDS is 5 years.
   D. The lower the blast percent, the longer is the survival rate.

Use the following information to answer questions 297–300.

A 53-year-old man reported to the laboratory for routine blood work as part of a yearly physical. He had been feeling tired for the last few months. Physical examination revealed splenomegaly. His CBC results are as follows: WBC 80.0×10^9/L ($80.0 \times 10^3/\mu L$); RBC 4.10×10^{12}/L ($4.10 \times 10^6/\mu L$); hemoglobin 123 g/L (12.3 g/dL); hematocrit 0.37 L/L (37.0%); platelet count 650×10^9/L (650,000/μL); differential count shows 40% polymorphonuclear neutrophils, 18% bands, 5% metamyelocytes, 7% myelocyte, 28% lymphocytes, and 2% monocytes. No RBC or WBC morphologic abnormalities are seen.

297. The peripheral blood findings are most consistent with a diagnosis of
   A. Neutrophilic leukemoid reaction
   B. Chronic myelogenous leukemia
   C. Acute myelogenous leukemia
   D. Regenerative left shift

298. Which of the following would yield the most diagnostic information for this patient?
   A. Sudan black B (SBB)
   B. Periodic acid–Schiff (PAS)
   C. Tartrate-resistant acid phosphatase (TRAP)
   D. Leukocyte alkaline phosphatase (LAP)

299. Which of the following myeloproliferative disorders is characterized by the presence of a t(9;22) chromosome abnormality and the BCR/ABL oncogene?
   A. Polycythemia vera
   B. Essential thrombocythemia
   C. Chronic myelogenous leukemia
   D. Chronic idiopathic myelofibrosis

300. How does the presence of this chromosome abnormality affect the prognosis?
   A. It is not a prognostic indicator.
   B. The prognosis is better when the abnormality is present.
   C. The prognosis is better when the abnormality is not present.
   D. Progression to acute lymphoblastic leukemia occurs more often when the abnormality is present.

answers & rationales

Hematopoiesis

1.

A. The need for oxygen delivery to developing tissues results in the production of erythrocytes before other blood cells. Erythropoiesis commences in the yolk sac as early as the fourteenth day of embryonic development. These primitive red cells produce embryonic hemoglobins that temporarily serve oxygen needs of the fetus. Myelopoietic and lymphopoietic activities begin when the liver and spleen become sites of production at 6–9 weeks of gestation; however, erythropoiesis still predominates. At this time, the red cells produce hemoglobin F, which is the chief oxygen carrier during fetal life.

2.

C. In the infant, there is an increased demand for blood formation because of the rate of growth. At birth, all bone marrow cavities are filled with hematopoietic tissue (active red marrow). As the growth rate slows, there is less need for active marrow. Fatty infiltration of the marrow becomes noticeable at about 4 years of age as cell production diminishes within the shafts of the long bones and is filled with yellow inactive tissue. Fat comprises 50% of the total marrow space in the adult. Except for lymphopoiesis, hematopoiesis is confined to the flat bones and pelvic area by the age of 25 years.

3.

C. Hematopoietic stem cells can make copies of themselves to maintain the stem cell pool and possess the ability to generate cells of all lineages (pluripotential). These stem cells give rise to multipotential myeloid and lymphoid progenitor cells, which ultimately produce progenitor cells that are restricted to a specific cell lineage. With appropriate cytokine stimulus, the committed progenitor cells undergo proliferation to recognizable precursors that produce an amplified number of mature end-stage cells. Stem cells and progenitor cells cannot be morphologically distinguished (look similar to small lymphocytes) but can be identified phenotypically by markers such as the stem cell marker CD34. CD34 expression is lost as antigens for a specific cell lineage are expressed. CD13 is a marker expressed by myeloid precursors.

4.

D. Unlike the infant, in which all bone marrow is capable of forming blood cells, the active marrow in an adult is confined to the flat bones of the skeleton such as the sternum and posterior iliac crest. Although the spinous processes of the vertebrae contain active marrow, these sites are rarely used for aspiration in adults because of the danger of damage to the spinal cord. Sternal puncture also presents a possibility of serious damage to underlying structures, but this site may be used because of easy accessibility or if the aspirate is a "dry tap" in the iliac crest. To obtain both a bone marrow aspirate and core biopsy, most marrow specimens are taken from the posterior iliac crest. The anterior iliac crest may occasionally be used in adults and sometimes the tibia in children less than 2 years of age.

5.

C. The ratio between all granulocytes and their precursors and all nucleated red cell precursors represents the myeloid-to-erythroid ratio. Myeloid precursors outnumber erythroid precursors by about 3 or 4 to 1 in the normal bone marrow. Although there are many more red blood cells in the peripheral blood than granulocytes, red blood cells have a much longer life span in circulation (120 days) as compared to granulocytes (about 8 hours). Granulocytes, therefore, require a more continual production than erythrocytes and are the most numerous marrow precursors. Alterations in the M:E ratio, such as 1:1 or 8:1, may indicate erythroid hyperplasia or granulocytic hyperplasia, respectively.

6.

B. A diverse group of growth factors (cytokines) regulate and maintain hematopoiesis in a steady state. Most hematopoietic growth factors are not lineage restricted but can act on more than one cell type and have multiple functions. For example, interleukins (IL-3) and colony stimulating factors (GM-CSF) affect multiple cell lines; whereas erythropoietin action is limited to erythroid cells. Cytokines are glycoproteins that usually express activity by binding to specific receptors on target cells. The action of growth factors on hematopoietic progenitor and precursor cells can stimulate or inhibit cell proliferation and differentiation as well as promote or suppress cell death. Growth factors may act alone or together to exert a positive or negative influence on hematopoiesis as well as on the function of mature cells. A determining factor for controlling the rate of cell production is cytokine stimulation in response to physiologic need.

7.

A. The liver of the fetus assumes responsibility for hematopoiesis about the second month of gestation. From 3 to 6 months of fetal development, the spleen, thymus, and lymph nodes are also involved, but the principal site of hematopoiesis remains the liver. By the seventh gestational month, the bone marrow becomes the primary hematopoietic site. Around birth, the liver and spleen have ceased hematopoiesis (except for splenic lymphopoiesis) but maintain the potential for reactivation of hematopoiesis.

8.

B. Erythropoietin (EPO) is a hormone that stimulates red cell production in the bone marrow by its action on the committed RBC progenitor cells. To maintain optimal erythrocyte mass for tissue oxygenation, the body's mechanism for sensing tissue oxygen levels is located in the kidney. Erythropoietin production increases when hypoxia is detected by renal oxygen sensors, with 90% being synthesized in the kidney and 10% in the liver. EPO levels in the blood vary according to the oxygen carrying capacity of the blood (e.g., EPO levels rise in anemia and fall when tissue oxygen levels return to normal).

9.

D. The marrow-derived common lymphoid progenitor cell ultimately gives rise to lymphocytes of T, B, or NK (presumably) cell lineages. Antigen-independent lymphopoiesis occurs in primary lymphoid tissue located in the thymus and bone marrow. The formation of immunocompetent T and B cells from precursor cells is influenced by environment (thymus, bone marrow) and several interleukins. Antigen-dependent lymphopoiesis occurs in secondary lymphoid tissue (spleen, lymph nodes, Peyer's patches of the gastrointestinal tract) and begins with antigenic stimulation of immunocompetent cells.

10.

B. Apoptosis is physiological cell death that can be induced by deprivation of growth factors or prevented by growth-promoting cytokines. Apoptosis plays an important role in the regulation of cell number and is deregulated in certain malignancies. Necrosis is accidental cell death by phagocytic cells and is associated with lethal physical damage. Cellular senescence describes cells that have lived their life span and will die of old age. Terminal differentiation refers to mature end-stage cells that are no longer capable of replication.

11.

A. Bone marrow consists of vessels, nerves, hematopoietic cells at various levels of maturation, and stromal cells encased in a membrane lining called the endosteum. The vascular system empties into a system of sinuses (venous sinusoids). A layer of endothelium lines these sinusoids. Blood cell formation occurs in hematopoietic cords located outside of the sinusoids and between the trabeculae of spongy bone. The bone marrow stroma (macrophages, adipocytes, fibroblasts, endothelial cells) forms an optimal microenvironment for developing cells by providing support and secreting cytokines. Mature differentiated cells can deform to penetrate the vessel wall and enter the sinuses and blood circulation.

12.

B. Bone marrow cellularity in the normal adult is approximately 50% hematopoietic tissue and 50% adipose tissue (fat), with a range of 30–70% cellularity being normocellular. Marrow cellularity is usually estimated from the core biopsy. An intact bone marrow can respond to demand by increasing its activity several times the normal rate if sufficient supplies and growth factors are available. The marrow becomes hypercellular when inactive fatty tissue is replaced by active hematopoietic marrow. In contrast, bone marrow failure may result in hypocellularity or aplasia with increased fat and a reduced number of hematopoietic cells.

13.

C. Interleukins and colony stimulating factors are cytokines produced by a variety of cells, including monocytes/macrophages, T lymphocytes, fibroblasts, and endothelial cells. It is essential that cytokines are continuously supplied by cells present in the bone marrow microenvironment during hematopoietic cell development, or cells will die. Erythropoietin functions as a true hormone because it is produced by the kidney, released into the blood, and carried to the bone marrow, where it stimulates red cell production.

14.

C. In a normal adult, the total blood volume is approximately 12 pints or 6 liters. Cells account for about 45% (44% is red cell mass) and plasma accounts for 55%. Alterations in red cell mass or plasma volume are reflected in the RBC count and in measurements of hemoglobin and hematocrit. True anemia or polycythemia is due to a decrease or increase in total RBC mass, respectively. A reduction in plasma volume with a normal RBC mass may cause relative (pseudo) polycythemia. Conversely, an increase in plasma volume with normal RBC mass may cause relative (pseudo) anemia.

15.

A. The pluripotent hematopoietic stem cell gives rise to lymphoid and myeloid progenitor cells. The lymphoid progenitor produces cells destined to become lymphocytic cells, whereas the myeloid progenitor cell produces progenitors committed to differentiation into granulocytic, erythrocytic, monocytic, or megakaryocytic lineages with appropriate stimulus. The cells produced by progenitor cells can be demonstrated using *in vitro* culture techniques; thus, the myeloid progenitor cell is termed CFU (colony forming unit)-GEMM based on the cell colonies formed.

16.

C. The mature megakaryocyte, the largest hematopoietic cell in normal bone marrow, has a multilobed nucleus and abundant, granular cytoplasm. Plasma cells are characterized by a round, eccentric nucleus and intensely blue cytoplasm. Osteoblasts and osteoclasts are non-hematopoietic cells that may be present in normal bone marrow. Osteoblasts are cells involved in bone formation that resemble plasma cells but are larger and often found in groups. Osteoclasts reabsorb bone and are similar to megakaryocytes in size but are multinucleated.

17.

D. In normal adult marrow, about 50% is fat, 40% is myeloid (granulocytic) cells, and 10% is erythroid cells. The M:E ratio is determined by performing a differential count of marrow precursor cells. The presence of 10% myeloblasts is an abnormal finding (reference range 0–2%), and a hematologic disease is likely. Megakaryocytes should be seen when scanning and are usually reported as normal, increased, or decreased in number. Marrow iron is assessed with Perl's Prussian blue stain, and it is normal to see stainable iron in macrophages, as well as iron granules in the cytoplasm of developing red cell precursors.

18.

B. The nucleus-to-cytoplasm ratio decreases as blood cell lines mature. With maturation, cells generally become smaller, the nuclear chromatin becomes clumpy and condensed, nucleoli disappear, and the cytoplasm loses its deep blue basophilia when stained with Wright's stain. Exceptions include megakaryocytes (because of endomitosis they grow larger as cytoplasm accumulates) and plasma cells (increased RNA and protein synthesis produces a deep basophilia).

19.

C. Thrombopoietin (TPO) is the major regulator of platelet production in the bone marrow by its action on committed progenitor and precursor cells of the megakaryocytic line. It is primarily produced by hepatocytes and possibly by the kidney. After marrow release, about 70% of platelets are in the blood circulation and 30% are sequestered in the spleen. Unlike erythropoietin, which is manufactured for routine therapeutic use, recombinant TPO is still being evaluated.

20.

B. Hematopoiesis within the medulla or inner part of the bone marrow is termed medullary or myeloid. Hematopoiesis that occurs in the liver and spleen (reactivation of fetal life) is called extramedullary or myeloid metaplasia (organs may enlarge). Cell production outside of the marrow space takes place when the bone marrow is unable to meet its production demands. This may occur in severe hemolytic anemias when the maximal capacity of the bone marrow to increase activity is exceeded. Myeloid metaplasia may also be an extension of a disease process such as myelofibrosis. Myelophthisis refers to the replacement of normal marrow hematopoietic tissue by fibrotic tissue or cancer cells, whereas myelodysplasia describes abnormal maturation of erythrocytic, granulocytic, and/or megakaryocytic cell lines. The period of intrauterine life when cell production occurs in the yolk sac may be termed mesoblastic.

Erythrocytes

21.

D. Normal red blood cells survive about 4 months, or 120 days. The entire life span of the red cell is spent inside the vascular tree, making it easier to determine the rate of production and destruction. Red cell survival depends upon an intact RBC membrane, sufficient cellular energy, and normal hemoglobin function. As red cells circulate for 120 days, enzymes are depleted and the ability to deform decreases. Under normal conditions, red cell loss due to aging (~1%) is equal to daily replacement. Most destruction of aged red cells occurs extravascularly by macrophages of the reticuloendothelial system (spleen, liver).

22.

B. The erythrocyte has a semipermeable membrane that allows water and some anions, such as chloride (Cl^-) and bicarbonate HCO_3^-, to enter the cell rapidly. Sodium ions (Na^+) enter the cell and potassium ions (K^+) leave the cell slowly but continuously. In order to maintain a high intracellular K^+ concentration and remove excess Na^+, ATP-dependent cationic pumps expel Na^+ and take in K^+. This regulation of intracellular cations allows the red cell to control its volume and water content.

23.

D. A molecule of hemoglobin is composed of four globular, protein subunits, and each subunit contains a heme group bound within a convoluted globin chain. Heme groups are identical and consist of protoporphyrin IX with a central iron atom, made largely in the mitochondria. Amino acids are sequenced on ribosomes to produce four types of globin chains (alpha, beta, delta, and gamma) that combine in identical pairs. A normal hemoglobin molecule consists of two alpha-globin chains and two non-alpha-globin chains, each of which binds a heme group. The different globin chains determine the hemoglobin type (A, A_2, or F).

24.

D. Culling is the process of removing aged or abnormal red blood cells from the circulation by the spleen. Red cells (7 μm) enter the spleen through the splenic artery and must squeeze back into active circulation through 2- to 4-μm clefts in the venous sinusoids. Aged or abnormally shaped red cells with impaired membrane flexibility are trapped in the splenic microcirculation and ingested by macrophages. The spleen is the largest filter of blood in the body and has an essential role in the "quality control" of red cells.

25.

C. Each hemoglobin molecule has four heme groups located at its surface, and oxygen binds to the central ferrous iron (Fe^{2+}) in heme. Deoxyhemoglobin (not carrying O_2) and oxyhemoglobin (carrying up to four O_2) are normal physiologic forms of hemoglobin with iron in the ferrous state. Hemoglobin in which the ferrous iron (Fe^{2+}) has been oxidized to the ferric state (Fe^{3+}) is known as methemoglobin and is unable to carry O_2. Carboxyhemoglobin is hemoglobin with carbon monoxide (CO) attached to ferrous iron rather than O_2. Both methemoglobin and carboxyhemoglobin are reversible.

26.

C. Of the total body iron present in a normal adult, approximately 70% is contained in hemoglobin (in red cells of the blood and marrow). Most of the remainder, ~25%, is found in storage sites as ferritin or hemosiderin. A much smaller amount of iron is contained in muscle myoglobin (4%) and respiratory enzymes such as peroxidase (1%). The structures of hemoglobin and myoglobin are similar (both consist of globin and heme), but myoglobin functions as an oxygen trap in the tissues.

27.

B. A senescent red blood cell is one that has lived its life span. Repeated passes through the spleen deplete the cells of glucose and decrease their surface area as membrane lipids are lost. The red cells are removed from the circulation by splenic macrophages that recognize subtle abnormalities in these cells, sequester them, and destroy them.

28.

B. "Poikilocytosis" is a general term that refers to deviations from the normal red cell shape (biconcave, discoid). "Anisocytosis" is the term used when differences in the sizes of red cells are described. Color in red cells is designated as normochromic (normal) or hypochromic (indicating a decreased hemoglobin concentration). Abnormally shaped red cells and red cell inclusions are associated with rigid red cells that have reduced deformability and shortened survival.

29.

A. Howell-Jolly bodies are nuclear (DNA) remnants that remain in the red cell after the nucleus has been extruded and may represent nuclear instability. These inclusions are associated with the defective nuclear maturation found in megaloblastic anemias and the rapid cell division that occurs in severe hemolytic anemias. Under normal circumstances, the spleen effectively pits these bodies from the cell. Pitting is a process that removes inclusions while leaving the rest of the red cell intact. It may be that the pitting mechanism is overwhelmed and cannot keep pace with inclusion formation in hemolytic anemias. Howell-Jolly bodies can also be seen in individuals after splenectomy who lack the normal pitting function.

30.

A. Spherocytes appear smaller and more densely staining than normal red cells and lack a central pallor area. Because they are the result of membrane loss, their surface area-to-volume ratio is decreased. Spherocytes should be distinguished from acanthocytes, which also lack a pallor area but have sharp, irregular projections. Echinocytes have a central pallor area and blunt, short projections. Red cells with intracellular rod- or bar-shaped crystals contain hemoglobin C crystals.

31.

B. The presence of lead causes an inhibition of several of the enzymes important in heme synthesis. Among these is pyrimidine 5′-nucleotidase, which is normally responsible for degradation of ribosomal ribonucleic acid (RNA). The lack of this enzyme apparently allows aggregates of incompletely degraded RNA to remain in the cell cytoplasm. It is this ribosomal material that appears on Wright's stain as punctate basophilic stippling. Precipitated hemoglobin forms Heinz bodies (not visible with Wright's stain), nuclear fragments are called Howell-Jolly bodies, and iron deposits are Pappenheimer bodies.

32.

D. The presence of red cell rouleaux (coining pattern) is a characteristic finding in multiple myeloma because of the increased concentration of immunoglobulins in the blood plasma (hypergammaglobulinemia). The excessive immunoglobulins are produced by malignant plasma cells. Cold hemagglutinin disease is characterized by red cell agglutination or clumping in a nonspecific pattern. "Hypersplenism" refers to an enlarged, overactive spleen that destroys both normal and abnormal cells, possibly causing pancytopenia.

33.

C. In viewing Color Plate 1 ■, the inclusions in the red blood cells are Howell-Jolly bodies. During passage through the microvessels of the spleen, the red cell is examined for intracellular inclusions or membrane-bound antibodies, which, if present, are removed. Abnormal red cells circulate longer, and inclusions such as Howell-Jolly bodies or Pappenheimer bodies will be seen post-splenectomy (or in conditions with splenic atrophy). The phagocytic removal of abnormal red cells is assumed by the liver, but the liver is not as efficient as the spleen. Howell-Jolly bodies are not associated with iron deficient or iron overload states.

34.

C. One of the reasons for increased intestinal absorption of iron is an accelerated rate of erythropoiesis (another is depletion of iron stores). Although the mucosal cell does act as a barrier in normal circumstances, this function is not absolute and controls break down in the presence of large amounts of iron, causing an excess to be absorbed. An acid pH is required for iron absorption, and sites of maximal absorption are the duodenum and upper jejunum. The body has no effective means for iron excretion.

35.

D. The presence of iron granules or deposits can be detected with Perl's Prussian blue iron stain. Siderocytes are mature red blood cells that contain stainable iron granules (abnormal). Sideroblasts are bone marrow nucleated red cells (normoblasts) that contain small amounts of iron in the cytoplasm (normal). Ringed sideroblasts are marrow normoblasts that contain iron in the mitochondria that forms a ring around the nucleus (abnormal). Siderocytes and ringed sideroblasts are associated with iron overload problems, particularly sideroblastic anemia. Reticulocytes may contain small amounts of unused iron that is normally removed by the spleen.

36.

C. A "shift to the left" in the oxygen dissociation curve of hemoglobin means that a higher percentage of hemoglobin will retain more of its oxygen at a given pressure. Thus affinity will be greater and oxygen delivery will be reduced. A higher or more alkaline pH and a lower temperature are associated with decreased oxygen dissociation. With conditions in the lungs (increased pH, decreased 2,3-BPG, decreased temperature), hemoglobin affinity for oxygen is increased, which favors oxygen uptake. With conditions in the tissues (decreased pH, increased 2,3-BPG, increased temperature), hemoglobin affinity for oxygen is decreased, which favors release of oxygen to the tissues.

37.

D. Erythropoietin (EPO) is a hormone produced by the kidney that increases erythropoiesis in the bone marrow in response to tissue hypoxia. The CFU-E (colony-forming unit–erythroid) is a committed erythroid progenitor cell with many receptors for erythropoietin. EPO stimulation of the CFU-E produces the recognizable pronormoblast and promotes differentiation of RBC precursors. The maturation time of erythrocyte precursors (5–7 days) can be reduced in times of increased need for red cells by the action of erythropoietin.

38.

C. Increased binding of 2,3-BPG (2,3-bisphosphoglycerate) decreases the affinity of hemoglobin for oxygen, which promotes oxygen release to the tissues, a compensatory mechanism in anemic patients. Increased pH (alkalinity) enhances oxygen affinity and thus inhibits delivery to the tissues. Less oxygen is available at higher altitudes, and this affects blood saturation and delivery to tissues. An increase in erythropoietin release will affect red cell production but does not have an immediate or direct impact on oxygen delivery.

39.

A. After the nucleus is extruded, reticulocytes spend about 2 days in the bone marrow before release into the blood, where maturation continues for another day. Intense erythropoietin stimulus can cause early release of bone marrow reticulocytes. These reticulocytes are larger and contain more filamentous reticulum than a more mature reticulocyte. These "shift" or "stress" reticulocytes exhibit diffuse basophilia on the Wright's stained smear and will need more than the usual 1 day in circulation to mature (to lose RNA). A very high number of reticulocytes in the blood circulation can increase the MCV. The level of reticulocyte maturity is best assessed by the immature reticulocyte fraction (IRF), an index reported by automated cell counters that is based on RNA content.

40.

C. Heinz bodies do not stain with Wright's stain and appear as "normal" hemoglobin even though their presence causes cell rigidity and membrane damage. They can be visualized on wet preps with phase microscopy or by using supravital stains, such as crystal violet or brilliant green. Heinz bodies consist of intracellular globin or hemoglobin precipitate that results from hemoglobin denaturation (G6PD deficiency, unstable hemoglobin variants) or excess globin chains (certain thalassemic syndromes). Basophilic stippling, Cabot rings, and Pappenheimer bodies are visible with both Wright's and supravital stains.

41.

B. The presence of schistocytes (schizocytes) on the smear indicates that red cells have been subjected to some form of physical trauma that causes damage. Red cell fragmentation can be the result of impact with fibrin strands, mechanical trauma by artifical surfaces, injury by heat, partial phagocytosis, or damage by toxins and drugs. Schistocytes are characteristic of the increased red blood cell destruction that occurs in severe hemolytic anemias but are not associated with anemias that result from defective bone marrow delivery of red cells to the blood.

42.

C. Tissue hypoxia associated with low erythrocyte and hemoglobin levels causes increased renal release of erythropoietin to stimulate bone marrow erythropoiesis. Depending on severity, the bone marrow responds by increasing its activity 6–8 times normal and becomes hypercellular because of an increase in RBC precursors (erythroid hyperplasia); and the M:E ratio falls. Nucleated red cells may be released into the blood along with the outpouring of reticulocytes. The number of nucleated red cells tends to correlate with anemia severity.

43.

C. Millions of hemoglobin molecules are produced in the red cell cytoplasm during maturation. When developing erythroid cells are deprived of essential hemoglobin components, the result is the production of microcytic, hypochromic red cells. It is thought that during maturation, extra cell divisions occur until a certain hemoglobin concentration is reached. Impaired hemoglobin synthesis may be the result of heme defects (involving iron or protoporphyrin) or may be caused by globin defects. Impaired DNA synthesis is associated with macrocytic red cells, and normocytic red cells are characteristic of enzyme defects.

44.

C. The nucleated red cells seen in Color Plate 2■ would be staged as orthochromic normoblasts (metarubricytes) when in the bone marrow. This is the last stage of red cell maturation that contains a nucleus. The pyknotic, degenerated nucleus is normally extruded out of the red cell in the marrow to yield the anucleate reticulocyte. The release of nucleated red cells into the blood before reaching maturity usually indicates a high demand for red cells.

45.

B. The red cell membrane consists of a protein shell heavily coated with lipids. The membrane lipid bilayer is maintained by constant interchange with plasma lipids. Acanthocytes are the result of abnormal plasma lipids that have altered the lipid composition of the membrane, often involving increased cholesterol content. Acanthocytes (spur cells) are associated with a congenital form of acanthocytosis and with liver disease, or are seen following splenectomy.

46.

A. The mature red cell, which lacks mitochondria and Kreb's cycle activity, depends on glucose metabolism for cellular energy. The end product of the anaerobic Embden-Meyerhof pathway (EMP) is ATP, which is necessary for membrane maintenance and volume control (cation pumps). The hexose monophosphate (HMP) pathway is aerobic and reduces oxidants by providing NADPH and glutathione. The Rapoport-Luebering shunt controls the amount of 2,3-bisphosphoglycerate that regulates hemoglobin affinity for oxygen. Oxidized hemoglobin (methemoglobin) is reduced to functional hemoglobin by the methemoglobin reductase pathway.

47.

C. The polychromatophilic normoblast (rubricyte) is the last red cell stage capable of mitosis. With cellular divisions, each pronormoblast produces up to 16 erythrocytes. The polychromatophilic normoblast is also the stage in which hemoglobin is first visible. The gray-blue color of the cytoplasm when Wright's stained is due to a mixture of hemoglobin and RNA, hence the name "polychromatophilic." The reticulocyte is the last stage able to synthesize hemoglobin.

48.

A. The major adult hemoglobin, Hb A, consists of two alpha- and two beta-globin chains. The switch from gamma chains (Hb F) to beta chains occurs 3–6 months after birth, and Hb A reaches adult levels (about 97%) around 6 months of age. Most globin chains produced in a normal adult are alpha and beta types (1:1 ratio) for hemoglobin A production. Hemoglobin A_2 contains delta chains and comprises about 2% of hemoglobin in normal adults. Epsilon chains are found in early embryonic hemoglobins only.

49.

C. Impaired DNA synthesis results in nuclear maturation that lags behind cytoplasmic development (asynchrony), decreased cellular divisions, and the production of macrocytic red cells. Defective nuclear maturation (megaloblastic) is almost always caused by a deficiency of vitamin B_{12} or folic acid, which are DNA coenzymes. Macrocytic red cells that are not due to vitamin B_{12} or folic acid deficiency (nonmegaloblastic) may be seen in liver disease or when reticulocytosis is pronounced. Microcytic, hypochromic red cells are the result of impaired hemoglobin synthesis.

50.

A. When iron is removed from the heme of destroyed red blood cells, it is bound to transferrin and recycled for hemoglobin production or goes to storage. The major storage form of iron is ferritin, which is a water-soluble iron complex bound in a protein shell called apoferritin. Hemosiderin is a water-insoluble complex of iron aggregates and protein that is derived from ferritin. The main site of iron stores is the liver, but storage iron is also found in the bone marrow and spleen.

51.

B. RBC indices are average values, so they have less meaning in heterogeneous RBC populations with wide size variations. Because the MCV is a mean red cell volume measurement, the presence of both microcytes and macrocytes would yield a falsely normal MCV value. One would expect the RDW (red blood cell distribution width) to be high because it is an index of variation in red cell size or anisocytosis. The RDW is low when red cells are of uniform size (a homogeneous population). The RDW is high when a heterogeneous population of red cells is present.

52.

B. Bilirubin is formed when hemoglobin degradation occurs in the reticuloendothelial system, primarily in the spleen. Unconjugated bilirubin is transported by the plasma to the liver, where it is conjugated. When excessive extravascular red cell destruction occurs, the plasma bilirubin level rises and is largely unconjugated bilirubin. When acute intravascular red cell destruction occurs, hemoglobin is released into the plasma and findings may include hemoglobinemia, hemoglobinuria, and hemosiderinuria.

53.

D. Haptoglobin forms a 1:1 complex with alpha-beta dimers of hemoglobin. The large size of this complex prevents filtration of the hemoglobin through the kidneys, where it can cause renal damage. Haptoglobin can be depleted in the plasma during major hemolytic events, such as malarial attacks, transfusion reactions, and other causes of severe intravascular red cell destruction.

54.

C. Codocytes (target cells) have an increased surface area-to-volume ratio and are associated with abnormal hemoglobin synthesis. They are found in hemoglobinopathies, especially hemoglobin S or C disorders, as well as thalassemias and iron deficiency. Target cells can also result from an increase in membrane lipids and may be seen in liver disease. Discocytes are normal biconcave red cells, and elliptocytes (ovalocytes) can be found in varying sizes. The teardrop shape of dacryocytes may occur when a red cell is stretched in the spleen and cannot regain its original shape.

55.

B. Heme synthesis begins in the mitochondria with the formation of aminolevulinic acid. Formation of the pyrrole ring structure occurs in the cytoplasm, resulting in the synthesis of coproporphyrinogen III. The final stages of porphyrin synthesis occur again in the mitochondria, culminating in the formation of heme when ferrous iron is incorporated into protoporphyrin IX in the presence of ferrochelatase.

56.

A. The red cell membrane consists of an outer bilayer of lipids with embedded, integral proteins and an underlying skeleton. Spectrin is the predominant skeletal protein that forms a cytoskeleton with other proteins, such as actin, protein 4.1, and ankyrin. The skeletal proteins are responsible for cell shape, deformability, and stability. Any defect in structure or extensive damage to the membrane cannot be repaired and may lead to premature red cell death.

57.

C. Reduced glutathione (GSH) counteracts oxidants that accumulate in the red cell. These occur as a result of normal metabolic activities and increase during infections or as a result of treatment by certain drugs. In the absence of GSH or as a result of enzyme deficiencies in the hexose monophosphate pathway (HMP), oxidant accumulation can lead to oxidation and precipitation of hemoglobin.

58.

C. Total iron-binding capacity (TIBC) represents the amount of iron that circulating transferrin could bind when fully saturated. In this test, the amount of transferrin protein in the serum is indirectly measured by adding ferric (Fe^{3+}) iron to the serum and allowing it to bind to the unsaturated sites on transferrin. Unbound iron is then removed and the sample analyzed for the remaining iron that is bound to transferrin. The serum iron level measures iron bound to transferrin. Under normal conditions, about one-third of the binding sites on transferrin are occupied with iron.

59.

B. The amount of circulating ferritin indirectly reflects the amount of storage iron in the tissues. A bone marrow exam is not essential to assess iron stores, except in complicated cases, because the serum ferritin test is considered a good indicator of iron storage status in most individuals. Because ferritin is an acute-phase reactant, it may be increased in chronic inflammatory disorders regardless of iron stores. Therefore, the serum ferritin should be interpreted with other iron tests. The percent transferrin saturation is the serum iron divided by the serum TIBC.

60.

B. Fetal hemoglobin can be distinguished from adult hemoglobin in red blood cells by the acid elution technique of Kleihauer and Betke. Only hemoglobin F remains in red blood cells after exposure to a citric acid–phosphate buffer solution at pH 3.3. Hb F has a higher oxygen affinity than Hb A (less binding of 2,3-BPG), so it carries oxygen well *in utero*. Hemoglobin F production decreases after birth, composing less than 1% of total hemoglobin in normal adults. In certain conditions (thalassemias, hemoglobinopathies), defective beta-chain production can be compensated for by increased production of gamma chains and formation of hemoglobin F (two alpha and two gamma chains).

Erythrocyte Disorders

61.

D. Deficiencies of folic acid (folate) and vitamin B_{12} result in abnormal DNA synthesis and a resultant delay in nuclear maturation in comparison to cytoplasmic development. These anemias are categorized as megaloblastic because of the giant red cell precursors observed in the bone marrow. The other anemias are characterized by defects of heme (sideroblastic anemia and iron-deficiency anemia) or globin synthesis (hemoglobin C disease).

62.

A. G6PD deficiency has a sex-linked inheritance pattern and is the most common enzyme deficiency in the hexose monophosphate (HMP) shunt. Individuals are asymptomatic unless exposed to oxidants, which compromise the ability of the glutathione reduction pathway to prevent the oxidation of hemoglobin. The oxidized hemoglobin precipitates in the form of Heinz bodies, which cause acute intravascular hemolysis. In the most common G6PD variant, the hemolytic episode is self-limiting, with old red cells that lack enzyme being destroyed and young red cells with some enzyme activity unaffected.

63.

B. Hemoglobin nomenclature indicates a number of things. The symbol α_2 or $\alpha_2{}^{A}$ indicates the presence of normal adult, or A, alpha chains. The designation $\beta_2{}^{26\ \mathrm{Glu} \rightarrow \mathrm{Lys}}$ indicates that lysine residues have replaced glutamic acid on position 26 of the beta chains. All types of E hemoglobin show a similar electrophoretic mobility and migrate closely to hemoglobins C and A_2 on cellulose acetate (alkaline pH). Hemoglobin E occurs with the greatest frequency in Southeast Asia.

64.

C. The peripheral blood as seen in Color Plate 3■ shows numerous elliptocytes (ovalocytes). If they were artifact due to smear preparation, they would be oriented in the same direction. Hereditary elliptocytosis (HE) is associated with symptomatic hemolytic anemia in only about 10–15% of the cases, but the presence of an enlarged spleen is evidence of ongoing extravascular destruction. In patients with chronic hemolysis, gallstones are a common complication because of excess bilirubin catabolism. In most persons with HE, anemia does not develop because bone marrow production of red cells compensates for the mild shortening of red cell life span.

65.

B. Cooley anemia, or beta-thalassemia major, would be the appropriate diagnosis in this case. In this condition, two beta-thalassemia genes are inherited that result in virtually no hemoglobin A production because no beta-globin chains are produced. The primary hemoglobin made is hemoglobin F. The severe microcytic anemia results from the destruction of red cell precursors in the bone marrow (ineffective erythropoiesis) and rigid red cells in the blood that contain unused alpha-globin chains. Nucleated red blood cells and target cells, as seen in Color Plate 4■, are common, as well as basophilic stippling. Infants with alpha-thalassemia major die *in utero* or shortly after birth. Hemoglobin H disease (three-gene deletion alpha-thalassemia) results from deficient, alpha-chain synthesis that leads to production of Hb H (four beta chains), an unstable hemoglobin that forms Heinz bodies and causes chronic hemolysis. No clinical manifestations are seen in patients with hereditary persistence of fetal hemoglobin (HPFH).

66.

C. As seen in Color Plate 5■, the presence of numerous target cells and SC crystals on the peripheral blood smear suggests the presence of hemoglobin SC disease. These bizarre crystals are distinguished by one or more blunt, finger-like projections that protrude from the cell membrane. Clinically, hemoglobin SC disease is not usually as severe as sickle cell disease, and electrophoresis shows equal amounts of Hb S and Hb C. Codocytes, in varying numbers, are typical of hemoglobin S, C, and E disorders.

67.

C. Pica is a clinical finding seen in some patients with iron deficiency. Pica is unusual cravings for nonfood items that may include dirt, clay, laundry starch, or, most commonly, ice. Among some cultures, pica is a custom (eating dirt) that may contribute to iron deficiency. In children, lead poisoning often results from the ingestion of dirt or lead-based paint from toys and may be related to iron deficiency. Porphyrias are a group of inherited disorders characterized by enzyme deficiencies and abnormal porphyrin metabolism. The presence of pyridoxine (pyridoxal-5'-phosphate or vitamin B_6) is important to early porphyrin synthesis.

68.

B. Folate deficiency is most commonly a result of poor dietary intake of folate alone or in combination with increased requirements as during pregnancy. Daily requirements for folate are high, and depletion of folate stores can occur within 4 months as compared to vitamin B_{12}, in which deficiency takes at least 2 years to develop (there are high stores). Thus, dietary deficiency of vitamin B_{12} is rare, but folate supplements are commonly required during pregnancy or in hemolytic anemias with excess cell turnover.

69.

C. The hemoglobin solubility test can detect the presence of hemoglobin S, which is insoluble in the dithionite reagent, whereas normal hemoglobin A is soluble. A positive screening test, however, does not distinguish between patients with hemoglobin AS trait, hemoglobin SC disease, and hemoglobin SS disease, so results must be confirmed by electrophoresis. Sickle cell trait is clinically asymptomatic with target cells only. Disorders prevalent in the malarial belt (sickle cell trait, G6PD deficiency, hereditary ovalocytosis, thalassemia minor) are thought to impart resistance to falciparum malaria. Repeated splenic infarctions by sickle cell masses in hemoglobin SS disease cause autosplenectomy by adulthood.

70.

D. The schistocytes in Color Plate 6■ are found in microangiopathic hemolytic anemia and caused by red cells shearing on fibrin strands deposited in small vessels. Widespread or localized (e.g., kidney) fibrin deposition in DIC, HUS, and TTP results in red cell fragmentation. In addition, thrombocytopenia is a usual feature of MAHA. ITP is characterized by severe thrombocytopenia that results from destruction of platelets by autoantibodies, but it is not associated with red cell damage or anemia.

71.

C. Ringed sideroblasts result from the accumulation of iron deposits in the mitochondria surrounding the nucleus of erythroid precursors. The deposits are secondary to a defect in heme synthesis and a pathological finding in sideroblastic anemia. Blocks in the protoporphyrin pathway required for heme synthesis may be hereditary (rare) or acquired and result in iron overload with increased marrow iron. Pappenheimer bodies and basophilic stippling are frequent findings on the blood smear, and increased serum iron, decreased TIBC, increased percent transferrin saturation, and increased serum ferritin are usual.

72.

D. Reticulocytosis is indicative of increased erythropoietic activity by the bone marrow. This is a normal response in conditions involving premature red cell destruction in the circulation or following blood loss due to acute hemorrhage. The reticulocyte count is consistently increased in active hemolytic disease because the marrow speeds up red cell production to supply replacement cells. Anemia develops when the rate of red cell destruction exceeds the marrow's ability to replace red cells (uncompensated hemolytic disease). The reticulocyte count is not usually elevated in pernicious anemia even though increased marrow erythropoiesis occurs. The defective cellular maturation that occurs in megaloblastic anemias results in the death of many red cells in the bone marrow (ineffective erythropoiesis).

73.

B. The major defect in hereditary stomatocytosis is altered permeability of the red cell membrane to Na^+ and K^+ ions. A net gain of sodium within the cell leads to increased water entry and the appearance of a swollen cell with a slit-like area of pallor. This is a heterogeneous group of disorders, in that a number of specific membrane defects have been postulated, and anemia varies from mild to severe. One autosomal dominant disorder is associated with Rh-null individuals.

74.

D. Polycythemia vera (PV) belongs to the group of disorders that are hematopoietic stem cell defects and commonly characterized as myeloproliferative disorders. Although the major increase in PV is in red blood cells, there is also an overproduction of granulocytes and platelets, particularly in the early stages of the disease. The increased production of red cells in PV is not due to the activity of erythropoietin. The production of erythropoietin is almost completely suppressed in this malignant condition.

75.

A. Aplastic anemia can be defined as blood pancytopenia resulting from bone marrow failure. This stem cell disorder results in a hypocellular marrow with few developing precursors and decreased production of all cell lines. The anemia is generally normocytic or slightly macrocytic with reticulocytopenia. The "defect" also affects resting hematopoietic cells in the liver and spleen, so extramedullary hematopoiesis does not occur to compensate for marrow failure.

76.

A. Because red blood cells and plasma are lost together, the hemoglobin and hematocrit will not reflect the severity of an acute hemorrhage until the lost blood volume begins to be replaced by the formation of plasma. The restoration of a normal blood volume is usually complete by 24 hours. It is then that the hemoglobin and hematocrit will reach their lowest point and will begin to rise only with the release of newly formed red cells, usually within 3–4 days.

77.

D. Elevated RBC, hemoglobin, and hematocrit values in a newborn are a carryover from intrauterine life, when a high number of red cells were needed to carry oxygen. Erythropoiesis is suppressed in response to the marked increase in oxygenation of tissues after birth, and the reticulocyte count, which is initially high, will fall along with a slow decline in the hemoglobin level. A hemoglobin value below 140 g/L (14.0 g/dL) is abnormal for a neonate. Newborn red cells are macrocytic and up to 10 nucleated red cells per differential may be seen.

78.

D. The red blood cells with single elongated projections, seen in Color Plate 7■, are dacryocytes or teardrops. Dacryocytes are often seen in disorders of marrow replacement that affect bone marrow architecture, especially myelofibrosis. Teardrops can also result from the splenic removal of inclusions and may be present in a variety of anemias. Drepanocytes or sickle cells are observed during a sickling crisis of sickle cell anemia. Acanthocytes, echinocytes, and/or codocytes can be found in liver disease (presence varies with disease severity).

79.

C. Myelophthisic anemia is an anemia of bone marrow failure. It is seen in patients who are experiencing bone marrow replacement of normal hematopoietic tissue by metastatic cancer cells, fibrosis, or leukemia. The anemia is considered a hypoproliferative anemia because there is no hemolysis involved and the cells are normocytic, normochromic. Disruption of the bone marrow by abnormal cells can result in the release of immature cells (nucleated red cells and immature neutrophils) into the blood and may involve blood cell production in extramedullary sites.

80.

A. Any idiopathic disorder is one for which there is no apparent cause. Ionizing radiation is a well-known cause of aplasia, as is chemical exposure (pesticides, benzene). Iatrogenic disorders are those that result from treatments for a different disorder; for example, aplasia can result from chloramphenicol treatment for bacterial disease. Aplastic anemia may develop as a complication from infections such as Epstein-Barr or hepatitis viruses.

81.

C. Diamond-Blackfan anemia is a congenital disorder that depresses only red blood cell production. Fanconi anemia is a congenital form of aplastic anemia that results in aplasia of all cell lines and has a high risk of developing acute myeloid leukemia or other cancers. The bone marrow distinguishes Diamond-Blackfan from the hypocellular marrow seen in aplastic anemia because there is a lack of erythroid precursors but a normal number of myeloid and megakaryocytic precursor cells.

82.

C. Dehydration is a cause of relative (pseudo) erythrocytosis due to plasma loss. High altitude adjustment, cardiac or pulmonary disease, and defective oxygen transport are all causes of absolute secondary erythrocytosis. Secondary erythrocytosis (polycythemia) is a compensatory increase in red cells, produced in an attempt to increase the amount of oxygen available to the tissues.

83.

B. In infants with homozygous alpha-thalassemia, no alpha-globin chains are produced (because of the deletion of all four alpha genes). Consequently, the infants have nearly 100% hemoglobin Bart's, which consists of four gamma-globin chains. This hemoglobin migrates farther toward the anode than Hb A. Because Hb Bart's has a very high oxygen affinity, it is useless for delivery of oxygen to the tissues, making its presence incompatible with life. Hemoglobin H, composed of four beta chains, also migrates farther than Hb A, but Hb H disease is not fatal.

84.

C. Bart's hydrops fetalis (homozygous alpha-thalassemia major) is a lethal condition in which all normal hemoglobins are absent and the presence of Bart's hemoglobin results in death due to hypoxia. Severe cases of Rh hemolytic disease of the newborn/fetus (erythroblastosis fetalis) are characterized by hemolytic anemia, high numbers of nucleated red blood cells, and hyperbilirubinemia that can cause brain damage (kernicterus). The bilirubin level is elevated but anemia is mild, if present, in ABO hemolytic disease of the newborn.

85.

B. The blood profile alone cannot distinguish folic acid and vitamin B_{12} deficiencies, because both are characterized by macrocytic ovalocytes, Howell-Jolly bodies, and hypersegmented neutrophils. Clinical severity generally differentiates the heterozygous (mild) and homozygous (severe) conditions of thalassemic and sickle cell syndromes. The anemia of acute blood loss is usually normocytic, whereas the anemia of chronic blood loss becomes microcytic due to the development of iron deficiency.

86.

C. The cause of the many spherocytes and polychromatophilic red cells seen in Color Plate 8■ would be best determined with the direct antiglobulin test (DAT). The differential diagnosis is hereditary spherocytosis (negative DAT) and warm autoimmune hemolytic anemia (positive DAT). Both of these hemolytic disorders are the result of membrane injury and would show an increased osmotic fragility result due to the spherocytes, elevated reticulocyte counts, and elevated urine urobilinogen, as well as elevated serum bilirubin levels.

87.

D. Schistocytes and spherocytes are associated with red cell destruction and would be found in clostridial septicemia (toxins), prosthetic heart valves (mechanical trauma), and thermal burns (heat). Microspherocytes can also result from the direct membrane damage caused by clostridial toxins and heat. Aplastic anemia is not a hemolytic anemia but is caused by decreased bone marrow production. Aplastic anemia is usually normocytic, with no evidence of red cell damage on the blood smear, and red cell destruction tests such as serum bilirubin would be normal.

88.

D. Erythrocytosis (polycythemia) is either absolute or relative. Absolute erythrocytosis occurs when the RBC mass increases, taking up a larger than usual proportion of the blood volume. Relative polycythemia occurs when the RBC mass stays normal but the amount of fluid volume decreases, thus increasing the proportion of the blood occupied by red cells as compared to the total blood volume which has decreased. Primary polycythemia is a condition of erythrocytosis without an underlying or contributing condition. The body produces an increased number of red cells without an increase in erythropoietin (an inappropriate response). Secondary polycythemia occurs when some underlying condition causes an increase in erythropoietin, so erythrocytosis occurs secondary to the condition (an appropriate response).

89.

A. Hemochromatosis is an excessive deposition of iron in body tissues that results in iron-laden macrophages, expansion of storage sites, and serious damage to organs (heart, liver). Iron overload can be hereditary or acquired as a complication of severe hemolytic anemias, frequent blood transfusions, or sideroblastic anemia. Hereditary hemochromatosis is caused by a mutation of the HFE gene that results in increased absorption of iron from the gastrointestinal tract and leads to iron overload. It is associated with low levels of hepcidin, an iron regulator, which causes increased iron absorption and release of iron from macrophages. The treatment for hereditary hemochromatosis is phlebotomy, and molecular testing is done for diagnosis.

90.

A. Abetalipoproteinemia, or hereditary acanthocytosis, is a rare autosomal recessive disorder of lipid metabolism. An absence of serum beta lipoprotein, a transport protein, causes abnormal plasma lipids. The numerous acanthocytes (spur cells) are the result of an alteration in the lipid content of the red cell membrane. The anemia is mild, but this disorder is associated with progressive neurologic disease.

91.

A. The majority of body iron is found in the hemoglobin of circulating erythrocytes. This means that any form of bleeding will lead to excessive iron loss. Iron balance is normally very tightly controlled through absorption rather than excretion. Iron deficiency in males is rare but, if present, it is usually the result of chronic gastrointestinal bleeding (ulcers, cancer).

92.

B. The severe transfusion-dependent anemia, which is typical of homozygous beta-thalassemia, is the result of imbalanced globin-chain synthesis and massive red cell destruction that far exceeds the rate of production. Decreased or absent beta chains lead to excess alpha chains that precipitate in red cells and subsequently are destroyed. The response is intense marrow erythroid hyperplasia, bone expansion, and erythropoiesis in extramedullary sites. A complication of continuous red cell hemolysis and repeated blood transfusions is iron overload. Patients require iron chelation therapy to prevent liver and heart failure. Splenectomy may be needed to reduce blood requirements, but it is not done before 4 years of age because of the increased risk of infection.

93.

A. Serum iron is low in both iron-deficiency anemia and the anemia of chronic disorders. The total iron-binding capacity (TIBC), which is an indirect measure of the amount of transferrin protein, is low in the anemia of chronic disease, whereas it is high in iron deficiency. Synthesis of transferrin is regulated by iron availability. Usually, when storage iron decreases, serum iron levels decrease and transferrin levels (TIBC) increase. In the anemia of chronic disorders, storage iron is normal or increased (but unavailable), and transferrin levels (TIBC) are decreased.

94.

C. Although the punctate basophilic stippling found in lead poisoning in erythrocytes is considered a classic finding, the anemia present is usually not severe unless accompanied by iron deficiency. The presence of lead inhibits several enzymes involved in the formation of heme, with a consequent increase in erythrocyte protoporphyrin and urinary aminolevulinic acid. The most significant effect of lead toxicity is the resulting neurological deficit and impairment of mental development.

95.

C. In Color Plate 9■, the dual population of red blood cells represented may also be termed dimorphic. This blood picture could be seen in a patient with microcytic, hypochromic anemia after the transfusion of normal red cells or when new normocytic, normochromic red cells are produced after successful treatment for iron deficiency. Concurrent deficiencies, such as coexisting iron and folate deficiency during pregnancy, would result in the production of both microcytic and macrocytic red cells.

96.

B. The cells visualized in Color Plate 10■ are sickle cells in the presence of target cells. The substitution of a valine for the glutamic acid normally found in the sixth position of the beta-globin chain causes red cells containing hemoglobin S to undergo the characteristic shape change that gives the sickle cell its name. A defect of both beta genes results in sickle cell disease, whereas a single gene mutation causes the sickle cell trait. Hemoglobin C results from the substitution of lysine for glutamic acid in the sixth position of the beta-globin chain.

97.

D. The number of irreversibly sickled cells (ISCs) and the proportion of S hemoglobin within the cells contribute collectively to the severity of sickle cell disorders. The classification of "trait" versus "disease" is not based on the severity of symptoms. The absence of Hb A and the presence of over 80% Hb S on electrophoresis would be classified as homozygous sickle cell disease (SS), whereas the heterozygous condition (AS) would show approximately 60% Hb A and 40% Hb S. Sickling is rare in the trait condition because of the lower concentration of Hb S. Sickle cell disease typically shows increased levels of compensatory Hb F, as does hemoglobin SC disease.

98.

B. Hyperbaric oxygen will reverse the sickling process, but it will also suppress erythropoietin, which stimulates the bone marrow to produce adequate replacement erythrocytes. Hydroxyurea reduces sickling by increasing Hb F levels and has been shown to improve the clinical course of patients plagued by painful crises. Treatment is primarily supportive and symptomatic, with efforts made to avoid those factors known to precipitate a crisis.

99.

D. In response to premature red cell destruction, the normal bone marrow can speed up red cell production. Hemolytic anemias typically have high reticulocyte counts, because the marrow can respond to the need for red cells. Generally, anemias caused by defective maturation or decreased production have inappropriately low reticulocyte counts, because the marrow fails to respond due to injury or lack of essential hematopoietic components. Low hemoglobin and hematocrit values reveal the presence of anemia but do not indicate *etiology. The reticulocyte count is particularly useful in distinguishing hemolytic anemias from other normocytic anemias that are not hemolytic.*

100.

C. Both iron deficiency and heterozygous thalassemia can present with a mild microcytic, hypochromic anemia. Target cells may be seen in both, but basophilic stippling is only found in thalassemia. The hemoglobin A_2 is normal in heterozygous alpha-thalassemia but is frequently twice the normal level in heterozygous beta-thalassemia, because these individuals compensate with increased delta-chain production due to deficient beta-globin chain synthesis. In this case, iron deficiency would likely be ruled out first with iron tests. Beta-thalassemia with hemoglobin S trait (Hb S/beta-thalassemia) produces a severe clinical picture similar to sickle cell anemia, with sickling of red cells.

101.

D. Oxidative denaturation is the primary mechanism of the hemolytic process. When glucose-6-phosphate dehydrogenase (G6PD) is deficient, the red blood cells cannot generate sufficient reduced glutathione (GSH) to detoxify hydrogen peroxide. Hemoglobin is oxidized to methemoglobin, denatures, and precipitates, forming Heinz bodies. The Heinz bodies cause the rigidity of the red cells, and hemolysis occurs as the cells try to pass through the microcirculation.

102.

B. In hereditary spherocytosis, the rigid spherocytes are being destroyed in the splenic microcirculation. Following splenectomy, the hemoglobin level should rise as the spherocytes circulate longer. Consequently, there is less need for increased red cell production by the bone marrow, and the number of reticulocytes released into the blood will fall. Approximately, 30% of platelets are normally sequestered by the spleen, so a transient increase in the platelet count occurs and red cell inclusions (Howell-Jolly and Pappenheimer bodies), normally pitted out of red cells by the spleen, will be observed.

103.

D. Unconjugated bilirubin levels will rise when either excessive intravascular or extravascular hemolysis is occurring. When hemolysis is intravascular, the free hemoglobin released into the circulation is bound by haptoglobin, and the complex is transported to the liver, where it is metabolized to bilirubin. Depletion of the haptoglobin protein will occur if use exceeds production, and then hemopexin binds hemoglobin for removal. When both haptoglobin and hemopexin are depleted, plasma hemoglobin levels will increase. The serum lactate dehydrogenase rises when red cells are broken down and intracellular LD enzymes are released.

104.

A. Hemolytic anemias can be classified by the mode of transmission (hereditary or acquired) and by the type of defect (intrinsic or extrinsic). With the exception of paroxysmal nocturnal hemoglobinuria (PNH), intrinsic defects are hereditary, and the defect that shortens survival is within the abnormal red cell. Hemolytic anemias due to extrinsic defects are acquired and caused by external agents or extracorpuscular factors that destroy the intrinsically normal red cell.

105.

C. Paroxysmal cold hemoglobinuria (PCH) is caused by an IgG biphasic antibody with P specificity known as the Donath-Landsteiner antibody. This autoantibody fixes complement to the red cells in the cold, and the complement-coated red cells lyse when warmed. PCH can be idiopathic or follow a viral infection and is characterized by acute intravascular hemolysis and hemoglobinuria after cold exposure. Cold autoantibodies usually show I specificity, whereas warm autoantibodies are often directed against Rh antigens on the red cells.

106.

B. The hypersegmented neutrophils and macrocytic ovalocytes seen in Color Plate 11■ suggest the presence of megaloblastic anemia. The two most common causes are lack of folic acid or vitamin B_{12}, which are coenzymes required for normal DNA synthesis. This patient's neurological symptoms are indicative of a vitamin B_{12} deficiency, because that vitamin is also needed for myelin synthesis (CNS).

107.

B. Pappenheimer bodies observed with Wright's stain can be confirmed with the Prussian blue stain and are composed of iron. The presence of siderotic granules in the red cells is associated with iron overload, and the serum ferritin test, which reflects the amount of storage iron, would be elevated. The test for parietal cell antibodies can be done to determine the cause of vitamin B_{12} deficiency.

108.

D. Hemoglobinopathies are a hereditary group of qualitative disorders in which genetic mutations cause the production of structurally abnormal globin chains. The three most common variant hemoglobins are Hb S, Hb C, and Hb E, all of which are due to an amino acid substitution in the beta-globin chain. Hemoglobin C is the second most common hemoglobin variant, after hemoglobin S, seen in the United States. Thalassemias are characterized by an absent or reduced rate of globin-chain synthesis.

109.

C. The structurally abnormal red cells in hereditary spherocytosis (HS) are deficient in spectrin and are abnormally permeable to sodium. The bone marrow produces red cells of normal biconcave shape, but HS cells lose membrane fragments and become more spherical as they go through the spleen and encounter stress in the blood circulation. The membrane defect is accentuated by the passage of red cells through the spleen, where they are deprived of glucose and are unable to generate sufficient ATP to pump sodium out of the cell. Ultimately, the red cells are trapped and destroyed in the spleen. The osmotic fragility is increased because of the membrane loss (reduced surface area-to-volume ratio), and the MCHC value may be increased.

110.

A. In hereditary elliptocytosis (HE), the red blood cells show increased permeability to sodium and may have one of several membrane defects linked to this heterogeneous disorder. These include deficiencies in skeletal proteins such as protein 4.1 or spectrin. The characteristic oval or elliptical shape is seen only in mature red blood cells, and it occurs in the circulation when HE red cells cannot return to a normal biconcave shape.

111.

C. The anemia of chronic disease (ACD) is very common and develops in patients with chronic infections (tuberculosis), chronic inflammatory disorders (rheumatoid arthritis, systemic lupus), and malignant disease (cancer, lymphoma). ACD has a complex etiology that includes impaired release of storage iron for erythropoiesis and a reduced response to erythropoietin. The anemia may be normocytic or microcytic, and severity depends on the underlying disorder.

112.

A. Hemoglobin C disease results from a homozygous substitution of lysine for glutamic acid at position 6 of the beta-globin chains. Numerous target cells and occasional intracellular C crystals will be found on the blood smear. Osmotic fragility is decreased (increased resistance) because of the many target cells, and electrophoresis will show an absence of Hb A and over 90% Hb C. The clinical severity of Hb CC is mild as compared to Hb SS or Hb SC diseases.

113.

D. Red cells that contain a high concentration of hemoglobin S will assume the sickle shape when deprived of oxygen (which can be reversed if reoxygenated). After repeated sickling, reversion capabilities are lost and irreversibly sickled cells (ISCs) are seen. Sickle cells are mechanically brittle, nondeformable cells that become impeded in circulation, causing blocks that restrict blood flow in vessels and leading to organs (vascular occlusive disease). They are easily trapped in the small vessels of the spleen, leading to obstructive ischemia and eventual destruction of splenic tissue.

114.

B. Aplastic anemia is bone marrow failure characterized by hypocellularity and decreased production of all cell lines. The normal M:E ratio (4:1) does not change in aplasia, because the number of both myeloid and erythroid precursors is decreased. In anemias such as sickle cell anemia, beta-thalassemia major, or megaloblastic anemia, the marrow becomes hypercellular because of an increase in erythroid precursors, and the M:E ratio falls.

115.

B. The immune hemolytic anemia indicated by the smear findings is warm autoimmune hemolytic anemia (WAIHA). Antibody-coated red cells are being partially phagocytized by macrophages (with receptors for IgG and complement), causing loss of membrane fragments. The spherocytes are ultimately destroyed, primarily in the spleen. The cause of the autoantibody production may be unknown (idiopathic), develop secondary to a disease that alters the immune response (chronic lymphocytic leukemia or lymphoma), or can be drug induced. Cold hemagglutinin disease is characterized by red cell agglutination due to a cold autoantibody.

116.

C. Intrinsic factor is a glycoprotein secreted by the parietal cells, along with HCl, that is needed to bind vitamin B_{12} for absorption. Pernicious anemia (PA), which is a megaloblastic anemia caused by the lack of intrinsic factor, is most common cause of vitamin B_{12} deficiency (cobalamin). PA is characterized by atrophy of the gastric parietal cells and achlorhydria (absence of HCl). Autoimmune factors are involved because a high percentage of patients produce autoantibodies to intrinsic factor (50%) and/or parietal cells (90%). The bone marrow erythroid precursors exhibit megaloblastic maturation, with nuclear maturation lagging behind cytoplasmic maturation (asynchrony is also seen in developing granulocytes and platelets). Many fragile red cells die in the bone marrow, and those released into the circulation have a very short survival, which causes a marked increase in lactate dehydrogenase levels.

117.

B. Hemoglobin D migrates in the same location as hemoglobin S on cellulose acetate at alkaline pH but does not cause sickling. The negative solubility test rules out the presence of hemoglobin S. Target cells are seen in large numbers in homozygous hemoglobin D disease. The quantification of 95% differentiates homozygous from heterozygous states where less than 50% hemoglobin D would be seen.

118.

B. G6PD deficiency compromises the ability of the glutathione reduction pathway to prevent the oxidation of hemoglobin. Oxidative stress may occur from infections, ingestion of mothballs, ingestion of fava beans, and certain drugs, including primaquine or sulfonamides. The oxidized hemoglobin precipitates in the form of Heinz bodies, which leads to a hemolytic crisis characterized by intravascular red cell destruction, removal of Heinz bodies by splenic macrophages, and the presence of spherocytes and fragmented red cells on the smear.

119.

B. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired defect of membrane structure in which red cells have a high affinity for complement binding. PNH is characterized by pancytopenia and chronic intravascular hemolysis with hemoglobinuria and hemosiderinuria. A stem cell mutation causes production of red cells, white cells, and platelets that are sensitive to complement lysis because of the loss of a membrane glycolipid (GPI). The sucrose hemolysis test can be used to screen for PNH red cells, but the Ham's acid serum test has been replaced by immunophenotyping for confirmation of PNH. Paroxysmal cold hemoglobinuria (PCH) is characterized by intravascular hemolysis and hemoglobinuria after cold exposure that is due to a complement-binding autoantibody. A transient finding of hemoglobinuria following forceful contact of the body with hard surfaces (as may be seen in joggers and soldiers) describes March hemoglobinuria.

120.

A. Secondary, acquired sideroblastic anemia is the result of blocks in the protoporphyrin pathway that can be identified and, therefore, are reversible if the toxin or agent is removed. Alcohol inhibits vitamin B_6 and anti-tuberculosis drugs also interfere with vitamin B_6 (pyridoxine). Lead poisoning causes multiple blocks in protoporphyrin synthesis, including inhibition of ferrochelatase, which is needed for iron incorporation into protoporphyrin to produce heme. Methotrexate is an antifolate chemotherapeutic agent that causes a drug-induced megaloblastic anemia.

121.

A. Although iron deficiency may be the most common cause of anemia in pregnancy, there is a mild form of anemia that develops during the third trimester in pregnant women with adequate iron levels. Although both erythrocytes and plasma increase during pregnancy, the plasma increases in a higher proportion, causing a relative (pseudo) anemia. This increased blood volume actually increases oxygen delivery to both the mother and the fetus.

122.

A. The anemia of chronic renal failure results from decreased production and release of erythropoietin from the diseased kidney. The drop in erythropoietin results in decreased red blood cell production by the marrow. Recombinant erythropoietin is of great value in treating anemia resulting from end-stage renal disease. Iron or folate supplements may be needed to maximize the response, especially in patients on dialysis. Uremic metabolites may cause reduced red cell survival and impairment of platelet function.

123.

D. Aplastic anemia is a stem cell defect that leads to decreased production of erythrocytes, leukocytes, and platelets (pancytopenia). The survival of red cells released into the circulation is normal. Infection is a serious problem because of the lack of neutrophils. The reduced number of platelets is responsible for the bleeding often seen. Treatment includes blood and platelet transfusions, antibiotics, growth factors, and steroids. Bone marrow transplantation may be necessary.

124.

B. The fish tapeworm competes for vitamin B_{12}, and a macrocytic (megaloblastic) anemia may develop. Hookworm infestation causes chronic blood loss and a microcytic anemia due to iron deficiency. A variety of organisms are associated with hemolysis, including malaria and clostridial infections. Viral hepatitis can cause marrow suppression and a normocytic, hypoproliferative anemia.

125.

A. A need for the increased oxygen carrying capacity provided by additional red blood cells is found in conditions such as pulmonary disease, where normal oxygenation is inhibited. A decrease in the ability of the cardiovascular system to appropriately circulate cells is another reason for increased erythrocytes. Individuals with a high level of methemoglobin, such as heavy smokers or persons with genetic disorders, cannot effectively unload oxygen. This results in a need for increasing the number of red blood cells to compensate. Renal tumors are associated with excess production of erythropoietin, leading to an inappropriate polycythemia.

126.

B. Thalassemias are a group of congenital disorders characterized by quantitative defects in globin-chain synthesis. Alpha-thalassemias result from gene deletions that cause a reduced rate of alpha-globin chain production. Beta-thalassemias result from point mutations that cause a reduced rate of beta-globin chain synthesis. Normally, equal amounts of alpha- and beta-globin chains are produced for Hb A synthesis. In alpha- or beta-thalassemias, synthesis of globin chains is imbalanced, because a decreased production rate of one type of globin chain causes an excess of the other (consequences will depend on the thalassemia type).

127.

C. Low serum iron and iron stores (represented by serum ferritin) characterize iron deficiency that is severe enough to result in anemia. The production of transferrin, the iron transport protein, increases as iron stores decrease. Transferrin saturation decreases dramatically so that transferrin is less than 15% saturated with iron.

128.

D. The switch from gamma-globin chain production for Hb F to beta-globin chain synthesis for Hb A occurs 3–6 months after birth. Clinical symptoms of a homozygous beta-globin chain defect, such as sickle cell disease or homozygous beta-thalassemia, will not be evident until about 6 months of age or shortly after. Alpha-globin chain production is normally high throughout fetal and adult life. A homozygous defect involving the alpha-globin chain will affect the infant *in utero*.

129.

A. The hemolytic crisis of malaria results from the rupture of erythrocytes containing merozoites. This event becomes synchronized to produce the fever and chill cycles that are characteristic of this infection. In severe infections, particularly those caused by *Plasmodium falciparum,* the massive intravascular hemolysis results in significant hemoglobinuria.

130.

D. The incorrect ratio of blood to anticoagulant caused the cells to shrink. This produced the crenated appearance of the red cells. This is an artifact as opposed to a significant clinical finding and can also be the result of prolonged blood anticoagulation. Spur cells (or acanthocytes) lack a central pallor area and have sharp projections, as opposed to crenated cells (or echinocytes), which have a pallor area and blunt projections.

131.

A. Pyruvate kinase (PK) is an enzyme of the Embden-Meyerhof pathway (anaerobic glycolysis). A deficiency of PK results in decreased ATP generation, which causes impairment of the cation pump and a loss of normal membrane deformability. PK-deficient cells have a shortened survival time, but clinical manifestations vary widely.

132.

B. When iron use exceeds absorption, iron stores (serum ferritin) are depleted first. At this early stage, there is no anemia (normal hemoglobin) and the transferrin level is normal. This is followed by increased transferrin synthesis (TIBC) and decreased serum iron. Finally, a microcytic, hypochromic anemia develops.

133.

A. In the anemia of chronic disease (ACD), a chronic illness causes impaired release of iron from storage. These patients have iron but are unable to use it for bone marrow erythropoiesis. Hepcidin, a hormone produced by the liver, plays a major role in the regulation of body iron by influencing intestinal absorption and release of storage iron from macrophages. Hepcidin levels increase during inflammation (positive acute-phase reactant), which causes decreased release of iron from stores. There is also impaired response of marrow red cell precursors to erythropoietin stimulation in ACD. The impaired response is thought to be related to the effects of inflammatory cytokines. Recombinant erythropoietin improves the anemia in some cases.

134.

C. In the idiopathic or primary type of sideroblastic anemia, the blocks in the protoporphyrin pathway (heme synthesis) that lead to iron overload are unknown and, therefore, are irreversible. The anemia is refractory (unresponsive) to treatment other than transfusion. Ringed sideroblasts and increased stainable iron will be found in the bone marrow when stained with Prussian blue. This primary, acquired form of sideroblastic anemia is also known as refractory anemia with ringed sideroblasts (RARS) and is classified as a myelodysplastic syndrome.

135.

B. The demand for red blood cell replacement in beta-thalassemia major during early childhood development results in a hyperproliferative marrow. Expansion of the marrow causes the bones to be thin and narrow. This may result in pathologic fractures. Facial bones have the Mongoloid appearance, with prominence of the forehead, cheekbones, and upper jaw.

136.

D. Cold autoimmune hemolytic anemia (CAIHA), or cold hemagglutinin disease, is characterized by the production of IgM cold autoantibodies that often show I specificity. The cause of the autoantibody production may be unknown (primary) or occur secondary to *Mycoplasma* pneumonia or lymphoma. Significantly high titers can result in agglutination of red cells in the extremities called Raynaud's phenomenon (acrocyanosis).

137.

A. The red blood cell distribution width (RDW) is an index of red cell size variation or anisocytosis. The RDW will be high when a heterogeneous cell population consisting of red cells with varying sizes is present (sickle cell anemia with compensation). The RDW is low when a homogeneous or single population of red cells is present that are of uniform size (thalassemia minor, anemia of chronic disease).

138.

D. Splenomegaly is a common finding in hemolytic anemias, because the spleen is the major site of extravascular red cell destruction. Patients with hereditary spherocytosis and hemoglobin SC disease often have enlarged spleens for this reason. Patients with beta-thalassemia major exhibit splenomegaly because of active splenic removal of red cells, but the spleen may also be a site of extramedullary erythropoiesis. Splenomegaly can also be due to extramedullary hematopoiesis in malignant disorders such as polycythemia vera or myelofibrosis. Splenomegaly would not be a characteristic finding in megaloblastic anemia.

Leukocytes

139.

C. The major function of leukocytes is defense, either by phagocytosis or by immune mechanisms. The phagocytic cells are the granulocytes and monocytes. The immune response is mediated by lymphocytes; however, monocytes play a role in immunity as antigen-presenting cells. Leukocytes may be classified according to granularity as granulocytes and nongranulocytes or divided based on nuclear segmentation as polymorphonuclears (PMNs) and mononuclears.

140.

D. Monocytes have a diameter up to 20 μm, making them the largest cells in the peripheral blood under normal conditions. Eosinophils and neutrophils have diameters of about 12 μm. The small lymphocyte is 8–9 μm in diameter, similar to the red blood cell, which has a diameter of 6–8 μm. Large lymphocytes range in size from 11 to 16 μm in diameter.

141.

A. After granulocytes are released from the bone marrow, they remain in the circulation one day or less. Their major function takes place in the tissues. They migrate through the vessel walls to reach areas of inflammation very soon after release. The life span of the granulocyte is short; however, eosinophils and basophils appear to survive longer in the tissues than neutrophils.

142.

C. Approximately 50% of the neutrophils in the peripheral blood are found in the circulating pool. This is the pool measured when a total WBC count is done. Another 50% are found adhering to vessel walls (marginal pool). These pools are in constant exchange. Emotional or physical stimuli can cause a shift of cells from the marginating pool to the circulating pool, causing a transient rise in the total WBC count. The total WBC count can double but returns to normal within several hours.

143.

A. Although some phagocytic activity has been attributed to the eosinophil, it is the segmented neutrophil and monocyte that have the greatest phagocytic activity. The neutrophil is the most important because of numbers and its ability to respond quickly, especially against bacterial pathogens. Monocytes arrive at the site of injury after the neutrophil to "clean up."

144.

B. The growth factor mainly responsible for regulating the production of granulocytes and monocytes is granulocyte/monocyte colony-stimulating factor (GM-CSF), which acts on the committed bipotential progenitor cell CFU-GM (colony-forming unit–GM). GM-CSF stimulation of granulocyte or monocyte production increases in response to need and can also affect the production of erythrocytic and megakaryocytic lineages. G-CSF induces granulocyte differentiation, and M-CSF supports monocyte differentiation. Erythropoietin (EPO) is a lineage-specific growth factor responsible for stimulating erythrocyte production, and thrombopoietin (TPO) is mainly responsible for regulating platelet production. Interleukins, particularly IL-3, influence multiple cell lines, including granulocytes and monocytes.

145.

A. The granulocyte mitotic pool contains the cells capable of division, which are the myeloblasts, promyelocytes, and myelocytes. The post-mitotic pool, or reserve, is the largest bone marrow pool and contains metamyelocytes, band and segmented forms. This pool is available for prompt release into the blood if needed (e.g., infection), and its early release is the cause of a "left shift." If released, the bone marrow mitotic pool can dramatically increase its activity to replenish this reserve (cytokine stimulation increases).

146.

B. A "shift to the left" means an increase in immature neutrophilic cells in the blood caused by bone marrow release of cells in response to infection or tissue damage. A redistribution of the blood pools because of emotional or physical stimuli is characterized by an increased WBC count without a left shift. A cell "hiatus" refers to a population of cells in which there is a gap in the normal maturation sequence. A cell hiatus is most often seen in acute leukemia, in which there are many blasts and a few mature cells but no intermediate stages.

147.

D. "Agranulocytosis" refers to an absence of granulocytes in both the peripheral blood and bone marrow. A deficiency of granulocytes is found in cases of aplastic anemia, in which deficiencies in red cells and platelets also occur. The early release of cells from the bone marrow will result in immature cells in the blood but is not referred to as agranulocytosis. Neutrophils that exhibit little or no granulation may be called hypogranular or agranular and are a sign of abnormal growth (dyspoiesis).

148.

B. Antibodies are synthesized by plasma cells, which are end-stage B lymphocytes that have transformed to plasma cells following stimulation by antigen. An end product of T cell activation is the production of cytokines (lymphokines) such as interleukins and colony-stimulating factors. T cells are surveillance cells that normally comprise the majority (about 80%) of lymphocytes in the blood. T cells regulate the immune response by helping (T helper or inducer cells) or suppressing (T suppressor cells) the synthesis of antibody by plasma cells.

149.

C. Absolute values for cell types are obtained by multiplying the percentage of the cell type by the total number of cells. In this case, $4000/mm^3 \times 0.65 = 2600/\mu L$ or $2.6 \times 10^9/L$. Although reference ranges vary, the normal absolute count for lymphocytes is from 1.0 to $4.0 \times 10^9/L$ and the normal percentage of lymphocytes is 20–44%. In this case, there is a relative lymphocytosis (increase in percentage), but the absolute lymphocyte value is normal. Percentages can be misleading, so the absolute number of a particular cell type should always be evaluated.

150.

B. Auer rods are seen in the cytoplasm of malignant cells, most often myeloblasts, and are composed of fused primary (nonspecific, azurophilic) granules. Hypersegmented neutrophils have five lobes or more and are associated with vitamin B_{12} or folate deficiency. Toxic granules are primary granules with altered staining characteristics that stain in late-stage neutrophils due to toxicity. Döhle bodies are agranular patches of RNA present in neutrophil cytoplasm and associated with toxic states.

151.

D. The total white blood cell count reference ranges for males and females are equivalent. WBC counts do change with age, being higher in newborns and children than in adults. Any change from basal conditions, such as exercise or emotional stress, will cause a transient leukocytosis due to a redistribution of blood pools. WBC values are lower in the morning and higher in the afternoon (diurnal variation).

152.

C. Neutropenia is associated with a risk of infection. The degree of neutropenia correlates with the infection risk from high susceptibility ($<1.0 \times 10^9/L$) to great risk ($<0.5 \times 10^9/L$). Infection increases with the degree and duration of the neutropenia. Shortness of breath and bleeding tendencies are clinical symptoms associated with severe anemia and thrombocytopenia, respectively.

153.

B. Basophils and tissue mast cells have receptors for IgE and complement components, which trigger degranulation when appropriate antigens are present and are responsible for severe hypersensitivity reactions (anaphylaxis). Basophils and tissue mast cells have morphologic similarities but represent distinct cell types. Basophils possess water-soluble granules that contain, among other substances, heparin and histamine (a vasodilator and smooth muscle contractor). Basophils have a segmented nucleus, and the granules, although often scanty, overlie the nucleus. The mast cell has a single round nucleus, contains many more granules than the basophil, and can be found in the bone marrow.

154.

C. The last stage in the granulocytic series that divides is the myelocyte. Cells before and including this stage constitute the bone marrow mitotic pool and undergo multiple cellular divisions. Nuclear chromatin progressively clumps and nucleoli are no longer present in the nondividing metamyelocyte stage that follows the myelocyte.

155.

C. The precursor cell that can first be recognized as granulocytic is the myeloblast and has no granules. Primary or nonspecific granule production begins and ends during the promyelocyte stage. The granules are distributed between daughter cells as mitotic divisions occur. Secondary or specific granule production begins with the myelocyte stage and continues during succeeding cell stages with the synthesis of products specific to the function of the particular granulocyte (neutrophil, eosinophil, or basophil).

156.

B. Eosinophils lack lysozyme, which is present in neutrophils and monocytes, and contain a distinctive peroxidase that differs biochemically from the myeloperoxidase of neutrophils and monocytes. Major basic protein is a component of the granules and is very important to the ability of eosinophils to control parasites. In addition, eosinophils play a role in modifying the allergic reactions caused by degranulation of basophils. Basophils release eosinophil chemotactic factor of anaphylaxis (ECF-A), which calls eosinophils to the site.

157.

A. Primary granules, which appear in the promyelocyte stage, may be called azurophilic or nonspecific granules. Specific or secondary granules (neutrophilic, eosinophilic, basophilic) appear in the myelocyte stage. Primary granules contain hydrolytic enzymes (e.g., myeloperoxidase, lysozyme, acid phosphatase) and are coated with a phospholipid membrane. Lactoferrin is a component of neutrophil granules. Primary granules are visible in the myelocyte stage, but in later stage cells the primary granules, although present, are less visible by light microscopy under normal conditions.

158.

A. The presence of toxic granules, Döhle bodies, and/or vacuoles in the cytoplasm of neutrophils (segmented, band, metamyelocyte, and myelocyte stages) is indicative of a neutrophilic response to inflammation. The changes observed in the "toxic" neutrophil may occur in patients with severe burns, some malignancies, exposure to toxic drugs and chemicals, and acute infection (most often bacterial). A Barr body is a "drumstick"-shaped body of nuclear material found in the neutrophils of females that represents the inactive X chromosome and is of no significance. Auer rods are seen in malignant myeloid cells, usually blasts. Hypersegmented neutrophils are associated with megaloblastic anemias but may be seen in long-term chronic infections. Pyknotic cells and vacuoles may be seen in overwhelming sepsis or in a degenerating blood specimen. Russell bodies are globular inclusions found in plasma cells that are composed of immunoglobulin.

159.

A. Diapedesis is the movement of cells (usually referring to neutrophils) from the blood stream into the tissues by squeezing through endothelial cells of the vessel wall. Chemotaxis is the movement of cells directed by chemotactic stimuli such as bacterial products, complement components, or injured tissue. Opsonization is the coating of an organism or foreign particle by IgG or complement for recognition and phagocytosis by neutrophils or monocytes. The ingestion of red cells, often coated with IgG or complement, is called erythrophagocytosis. Margination is the attachment of neutrophils to the endothelial lining of the blood vessels.

160.

D. Basophil granules contain histamine, a potent vasodilator and smooth muscle contractor, that is responsible for the systemic effects seen in immediate hypersensitivity reactions (type I), which are also termed anaphylaxis. Degranulation occurs when basophils are coated with an IgE type of antibody that recognizes a specific allergen, such as bee venom, certain plant pollens, or latex. The resulting anaphylactic shock can be life threatening.

161.

B. Morphologic criteria such as cell size, nuclear shape, and chromatin pattern or cytoplasmic granularity cannot be used to identify lymphocyte subtypes. Monoclonal antibodies (CD surface markers) to specific surface and cytoplasmic antigens can distinguish lymphocyte subpopulations and identify the development stage. For example, blood lymphocytes that are B cells express CD19 and CD20 markers, T cells express CD2 and CD3 (and either CD4 or CD8 markers), and NK cells express CD56. Natural killer cells often exhibit large granular lymphocyte morphology (LGLs).

162.

B. Plasma cells are the mature end stage of the B lymphocyte, producing immunoglobulins (antibodies) in response to activation by a specific antigen (humoral immunity). The antibody produced by a single plasma cell is of one immunoglobulin type. Natural killer (NK) cells recognize and kill tumor cells or cells infected with virus through direct contact. Virocytes are reactive lymphocytes, and thymocytes are immature T cells. T lymphocytes provide cellular (cell mediated) immunity.

163.

A. A function of the eosinophil is to modify the severe allergic reactions caused by degranulation of the basophil. Neutrophils have receptors for the opsonins IgG and complement and are the most important cell in the initial defense against acute bacterial infection. Neutrophils are nonspecific phagocytes, ingesting bacteria, fungi, dead cells, etc., and they contain hydrolytic enzymes, including muramidase (lysozyme) and alkaline phosphatase. Neutrophils die in the performance of their function and are removed by macrophages.

164.

C. The nucleus in both monocytes and reactive lymphocytes can be irregular in shape, with indentations, although a monocyte nucleus often has folds and lobulations. Reactive lymphocytes characteristically have an increased amount of dark blue cytoplasm, whereas monocyte cytoplasm is usually a blue-gray color. Lymphocytes lack the many fine granules that give monocytes a typical "ground glass" appearance of the cytoplasm, but monocytes can occasionally have larger granules. Sharp indentation of the cytoplasm by adjacent red cells and an increased number of large granules are features of reactive lymphocytes. Vacuoles, although more commonly present in monocytes, can also be seen in reactive lymphocytes.

165.

B. Indentation of the nucleus (kidney shape) is the feature that characterizes the metamyelocyte stage. Specific granules begin forming in the myelocyte and persist through later stages. Cytoplasmic color is not a reliable feature, because it is variable and may not differ significantly from the myelocyte or band stage. Nucleoli are absent in metamyelocytes and may not be visible in myelocytes (they may be indistinct).

166.

A. Young children have the highest peripheral lymphocyte concentrations, ranging from 4.0 to 10.5×10^9 cells/L at 1 year of age and declining to $2.0–8.0 \times 10^9$ cells/L by 4 years of age. Lymphocyte counts decrease with age because of a decrease in lymphocyte stimulation and processing of antigens, ranging from 1.0 to 4.0×10^9 cells/L in adults. In addition to the difference in lymphocyte number in children, the normal morphology of children's lymphocytes differs from that of adults. Patient age should be considered when deciding between normal and abnormal lymphocytes.

167.

A. Early B cell precursors would be expected to express TdT, CD10, and CD34. TdT, the enzyme marker for terminal deoxynucleotidyl transferase, and the stem cell marker CD34 are present on the earliest B or T lymphoid cells. Surface immunoglobulin (SIgM) can only be detected on B cells at later stages of development. TdT can be used to differentiate the leukemic cells of acute lymphoid leukemia from acute myeloid leukemia. CALLA (CD10 or common ALL antigen) is a marker found in precursor types of B cell ALL.

168.

C. Acid hydrolases and the number of lysosomes increase as the blood monocyte matures into a tissue macrophage. Macrophages are widely dispersed in body tissues and organs of the reticuloendothelial (RE) system (also known as the mononuclear phagocyte system). Macrophages have receptors for IgG and complement, and they serve as phagocytes by ingesting debris and dead cells (usually neutrophils) at sites of inflammation. Macrophages act in the immune response as antigen-presenting cells by ingesting and exposing antigens for recognition by lymphocytes. Monocytes/macrophages secrete complement components and cytokines, including colony stimulating factors and interleukins.

169.

B. Antigen-*independent* lymphopoiesis occurs in primary lymphoid tissue located in the thymus and bone marrow. The formation of immunocompetent T and B cells from the lymphoid progenitor cell is influenced by environment (thymus, marrow) and several interleukins. Antigen-*dependent* lymphopoiesis occurs in secondary lymphoid tissue (spleen, lymph nodes, Peyer's patches) and begins with antigenic stimulation of immunocompetent T and B cells. Lymphocytes are the only white cells that recirculate (i.e., return to the blood from the tissues).

170.

D. Myeloperoxidase is an enzyme present in the primary granules, regardless of the phagocytic activity of the cell. The products produced during the respiratory burst, many of them short lived, are generated in response to chemotactic activation and ingestion of microbes. Generation of oxygen metabolites is necessary for microbial killing.

Leukocyte Disorders

171.

C. The Epstein-Barr virus (EBV) attaches to receptors on B lymphocytes, and the virus is incorporated into the cell. The infection generates an intense immune response of T cells directed against infected B cells. It is the activated T lymphocytes that comprise the majority of reactive lymphocytes seen in the blood of patients with infectious mononucleosis. Other B cells produce nonspecific polyclonal (heterophile) antibody in response to the EBV infection.

172.

C. The malignant cells of hairy cell leukemia (HCL) stain positive with acid phosphatase in the presence of tartaric acid; that is, hairy cells contain tartrate-resistant acid phosphatase (TRAP). Normal cells stain acid phosphatase positive, but staining is inhibited by the addition of tartrate. HCL is a chronic disorder, mainly confined to the elderly. The spleen usually shows marked enlargement, but enlarged lymph nodes are very uncommon. Hairy cells are malignant B cells, and pancytopenia is usual at presentation.

173.

A. The lymphoid cells of B cell acute lymphoblastic leukemia (FAB type L3) are morphologically identical to the malignant B cells of Burkitt lymphoma (large cells with basophilic cytoplasm and cytoplasmic lipid vacuoles). Although the site of origin is the bone marrow in B cell ALL and the tissues in Burkitt lymphoma, the World Health Organization (WHO) classifies them as the same disease entity with different clinical presentations (Burkitt leukemia/lymphoma). Both chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) are malignant proliferations of small, mature lymphocytes, and diagnosis is based on the predominant site of involvement. Mycosis fungoides and Sézary syndrome are different stages of a cutaneous T cell lymphoma in which the skin is the early site of involvement, with subsequent progression to the bone marrow and blood.

174.

D. A leukoerythroblastic blood profile, which refers to the presence of both immature neutrophils and nucleated red cells, is most commonly associated with conditions involving bone marrow infiltration by malignant cells (leukemia, cancer) or replacement by fibrotic tissue. A neutrophilic left shift is defined as the presence of increased numbers of immature neutrophils in the blood without nucleated red cells. A regenerative left shift and a neutrophilic leukemoid reaction are characterized by varying degrees of leukocytosis and a neutrophilic left shift, most often found in response to infection. In contrast, a degenerative left shift refers to leukopenia and a left shift that may occur if marrow pools are depleted in an overwhelming infection (use exceeds the bone marrow's capacity to replace).

175.

A. "True" Pelger-Huët anomaly is a benign autosomal dominant trait characterized by hyposegmentation of the granulocytes, coarse nuclear chromatin, and normal cytoplasmic granulation. The cells have no functional defect. It is of practical importance to recognize this anomaly so that it is not confused with a shift to the left due to infection. Acquired or "pseudo" Pelger-Huët is commonly associated with myeloproliferative disorders, myelodysplastic syndromes, or drug therapy. *Pelgeroid cells* are hyposegmented and the cytoplasm is frequently hypogranular.

176.

A. Eosinophils are decreased in Cushing syndrome, in which the adrenal glands secrete large amounts of adrenocorticosteroids. Eosinophils are increased in allergic disorders, various skin diseases, and certain types of parasitic infections (especially those due to intestinal and tissue-dwelling worms). Eosinophilia is also seen in chronic myelogenous leukemia and Hodgkin lymphoma.

177.

D. Chronic granulomatous disease (CGD) is a hereditary disorder in which neutrophils are incapable of killing most ingested microbes. The disease is usually fatal because of defective generation of oxidative metabolism products, such as superoxide anions and hydrogen peroxide, which are essential for killing. Chemotaxis, lysosomes, phagocytosis, and neutrophil morphology are normal. Several variants of CGD have been described, with specific enzyme defects and different modes of inheritance. The more common type of CGD has a sex-linked inheritance pattern.

178.

B. A drug-induced megaloblastic blood profile with macrocytic ovalocytes and hypersegmented neutrophils is shown in Color Plate 11■. This is a common finding in patients receiving antifolate chemotherapeutic drugs such as methotrexate. Recombinant erythropoietin is associated with a reticulocyte response and used to treat a variety of conditions, such as renal disease, anemia of chronic disease, or anemia caused by chemotherapy. Chloramphenicol is an antibiotic with a known association for aplasia due to marrow suppression.

179.

C. Primary or essential thrombocythemia (ET) is a chronic myeloproliferative disorder in which the main cell type affected is the platelet. An extremely high number of platelets are produced, but abnormal platelet function leads to both bleeding and clotting problems. The bone marrow shows megakaryocytic hyperplasia. The hemoglobin value and platelet count are increased in polycythemia vera, and CML is characterized by a high WBC count. Malignant thrombocythemia must be differentiated from a reactive thrombocytosis seen in patients with infection or following surgery. In reactive causes, the platelet count is rarely over 1 million $\times 10^9$/L, platelet function is normal, and thrombocytosis is transient.

180.

B. The bone marrow is progressively replaced by fibrotic tissue in myelofibrosis, a chronic myeloproliferative disorder. Attempts to aspirate bone marrow usually result in a "dry tap." A biopsy stain demonstrates increased fibrosis (fibroblasts are thought to be stimulated by megakaryocytes). The presence of teardrop-shaped red blood cells is an important feature of myelofibrosis. In addition, abnormal platelets, a leukoerythroblastic blood profile and myeloid metaplasia in the spleen and liver are often associated with this disease. A high LAP score (reference range 13–160) and increased RBC mass are found in polycythemia vera, but the LAP score is low in chronic myelogenous leukemia.

181.

B. A striking lymphocytosis may be seen in children with pertussis, but normal lymphocytes, rather than reactive lymphocytes, are present. A relative and/or absolute lymphocytosis with reactive lymphocytes in various stages of activation, as seen in Color Plate 12■, is characteristic of infection caused by Epstein-Barr virus (EBV), cytomegalovirus (CMV), and toxoplasmosis. A positive heterophile antibody test can help distinguish infectious mononucleosis caused by EBV from conditions with a similar blood picture. Epstein-Barr virus is also linked to Burkitt and Hodgkin lymphomas.

182.

A. B cell chronic lymphocytic leukemia (CLL) is by far the most common type found in the United States. Immune dysfunction because of hypogammaglobulinemia occurs in later stages of the disease, as does thrombocytopenia. Development of warm autoimmune hemolytic anemia is a frequent occurrence in patients with CLL. Treatment for B cell CLL is conservative and aimed at controlling symptoms. T cell CLL is a rare and is a more aggressive disease.

183.

D. Waldenström macroglobulinemia is caused by a proliferation of transitional B lymphocytes (lymphoplasmacytic or plasmacytoid lymphs) that secrete high amounts of monoclonal IgM. Because IgM is a macroglobulin, blood hyperviscosity is the cause of many of the symptoms found in this disease (bleeding and visual impairment). Plasmapheresis can reduce the IgM protein concentration. Hepatosplenomegaly is common in Waldenström disease (rather than bone lesions).

184.

C. The elevated WBC count and toxic neutrophils seen in Color Plate 13■ indicate an extreme response to severe infection (bacterial septicemia, fungal) or treatment with recombinant myeloid growth factors. GM-CSF and G-CSF are used to increase cells for peripheral stem cell transplant and reduce infection in patients after high-dose chemotherapy or during transplant. A leukemoid reaction is one that mimics the type of blood picture seen in leukemia. It is associated with extremely high leukocyte counts (often greater than 50×10^9 cells/L) and is usually found in severe infection. The most common type of leukemoid reaction is neutrophilic, but lymphocytic leukemoid reactions also occur. HIV infection is associated with leukopenia and lymphocytopenia.

185.

B. The blood shown in Color Plate 14■ is from an elderly patient with chronic lymphocytic leukemia (CLL), which is characterized by an absolute lymphocytosis and a predominance of small, mature lymphocytes with hyperclumped nuclear chromatin. Elevated leukocyte counts are usual, as are fragile, smudged lymphocytes. Acute lymphoblastic leukemia (ALL) typically occurs in children and is characterized by immature lymphoid cells. Plasmacytoid lymphocytes and red cell rouleaux may be found in the blood of individuals with Waldenström disease. Viral infections are associated with a lymphocytosis and the presence of reactive lymphocytes that are heterogeneous in morphology. Reactive lymphocytes exhibit a variety of forms with regard to size and cytoplasmic staining intensity as compared to the homogeneous cell populations present in malignant disorders such as CLL and ALL.

186.

D. Progression to acute leukemia is a very unlikely event for patients with chronic lymphocytic leukemia, even though there is no cure. Patients with chronic myelogenous leukemia typically progress to "blast crisis," most often of myeloid type, unless treated with imatinib mesylate (Gleevec®) in the chronic phase. Refractory anemia with excess blasts (RAEB) is the most likely type of myelodysplastic syndrome to develop acute myelogenous leukemia. Refractory anemia with ringed sideroblasts (RARS) is "preleukemic" but fairly stable.

187.

A. Gaucher disease is a lipid storage disorder in which there is an accumulation of glucocerebroside in the macrophages because of a genetic lack of glucocerebrosidase, an enzyme required for normal lipid metabolism. Gaucher cells are found in the liver, spleen, and bone marrow. Niemann-Pick disease is caused by a deficiency of sphingomyelinase in which "foamy" macrophages, called Niemann-Pick cells, are filled with sphingomyelin. Normal macrophages may contain iron and other cellular debris.

188.

D. The presence of Reed-Sternberg cells is the diagnostic feature of Hodgkin disease (lymphoma). The Reed-Sternberg giant cell is usually binucleated, and each lobe has a prominent nucleolus. Studies suggest that this neoplastic cell is of B cell lineage. It is not found in the blood but only in the tissues. Circulating T cells with a convoluted nucleus describe the Sézary cells seen in Sézary syndrome, the leukemic phase of mycosis fungoides. A monoclonal population of large lymphoid cells or immature B cells with nuclear clefts is most descriptive of lymphoma cells, present in certain types of peripheralized non-Hodgkin lymphoma, that have spread from the tissues to the bone marrow and blood.

189.

B. The overall reaction of the body to tissue injury or invasion by an infectious agent is known as inflammation. This response brings leukocytes to the site of infection or tissue damage and is associated with activation of inflammatory mediators, including cytokines (IL-1), molecules released by cells (histamine), and byproducts of plasma enzyme systems (complement, kinins, fibrin). The plasma concentration of positive acute-phase reactants (APRs), such as C-reactive protein (CRP) and fibrinogen, can increase dramatically in response to inflammation, and levels of albumin and transferrin will fall (negative APRs). The erythrocyte sedimentation rate (ESR) will be elevated, primarily because of the rise in fibrinogen.

190.

C. The abnormal cells found in acute promyelocytic leukemia (FAB type M3) contain large numbers of azurophilic granules. These granules contain procoagulants that on release hyperactivate coagulation, resulting in disseminated intravascular coagulation. Although other acute leukemias may trigger DIC, M3 is the one most frequently associated with this life-threatening bleeding complication. If DIC is resolved, many patients with acute promyelocytic leukemia respond favorably to therapy with retinoic acid, which causes maturation of the malignant promyelocytes. The presence of t(15;17) has diagnostic and prognostic significance, and acute promyelocytic leukemia is classified with "acute myeloid leukemias with recurrent cytogenetic translocations" by the World Health Organization (WHO). Acute myeloblastic leukemia with t(8;21) is also included in this WHO category (correlates with FAB type M2).

191.

D. Although a hallmark of acute lymphoblastic leukemias (ALL), lymphadenopathy is not associated with acute myelogenous leukemias. ALL is also more likely to have central nervous system involvement, and the CNS is a potential site of relapse. Hepatomegaly and splenomegaly are associated with both types of acute leukemia, as well as with the presence of anemia, neutropenia, and thrombocytopenia. Common presenting symptoms are fatigue, infection, or bleeding. If untreated, both acute myelogenous and lymphoblastic leukemias have a rapidly fatal course.

192.

B. The blast cells shown in Color Plate 15■ are from a child with CALLA positive, precursor B acute lymphoblastic leukemia. The malignant cells would be expected to express CD10, the common ALL antigen marker; the B cell lineage marker CD19; and TdT (terminal deoxynucleotidyl transferase), a marker on early lymphoid cells. Precursor T acute lymphoblastic leukemia would express TdT and the T cell markers CD2 and CD7. CD13 and CD33 are myeloid markers, and CD14 is a marker for monocytic cells.

193.

A. The "packed" bone marrow with predominantly immature blast cells and few normal precursor cells, as seen in Color Plate 16■, is most indicative of a patient with acute leukemia. Although chronic leukemias usually have a hypercellular marrow, the malignant cells are more mature or differentiated (i.e., able to mature beyond the blast stage). Myelodysplastic syndromes are associated with a hypercellular bone marrow, but the marrow blast percent is less than 20% (using WHO criteria). Aplastic anemia is characterized by a hypocellular bone marrow with few cells.

194.

C. The secretion of large amounts of monoclonal IgG or other immunoglobulin light chains by a malignant clone of plasma cells produces a characteristic M spike on serum and urine protein electrophoresis. In some cases, only the light chains are produced in excess. Because the light chains are easily cleared by the kidneys, they may appear only in the urine (Bence-Jones proteinuria). Renal impairment in multiple myeloma is associated with the toxic effects of filtered light chains. High levels of serum beta microglobulin correlate with the myeloma tumor burden. Cryoglobulins are proteins that precipitate in the cold and may be seen in multiple myeloma and Waldenström macroglobulinemia.

195.

C. Multiple myeloma is a malignant lymphoproliferative disorder characterized by a clonal proliferation of plasma cells and multiple bone tumors. Myeloproliferative disorders are characterized by a proliferation of bone marrow cells (granulocytic, monocytic, erythrocytic, megakaryocytic), with usually one cell type primarily affected. For example, the main cell type affected in polycythemia vera is the erythrocyte, and the platelet is mainly affected in essential thrombocythemia. Transformation among the myeloproliferative disorders is frequent.

196.

B. The Philadelphia chromosome, t(9;22), is detected in almost all cases of CML (depends on detection method) and results in a mutated BCR/ABL fusion gene. The resulting fusion protein causes increased tyrosine kinase activity, which promotes cell proliferation. Imatinib mesylate (Gleevec®) is a therapeutic agent that targets the molecular defect by blocking tyrosine kinase activity and is now a first-line drug used in the chronic phase of CML. The t(15;17) that is diagnostic of promyelocytic leukemia results in a PML/RARA (retinoic acid receptor alpha) fusion gene that blocks maturation. Many PML patients respond to retinoic acid therapy, which induces promyelocyte differentiation. Nearly all cases of Burkitt lymphoma have t(8;14), which is a translocation of the MYC gene from chromosome 8 to the Ig heavy chain (IgH) region on chromosome 14. JAK2 (Janus kinase) is a point mutation in a gene regulating cell proliferation, and it is present in over 90% of polycythemia vera cases and approximately 50% of those with essential thrombocythemia and myelofibrosis. Detection of cytogenetic and molecular mutations has diagnostic and prognostic significance and is an important tool in monitoring response to treatment.

197.

B. The French-American-British (FAB) classification of acute leukemias, myeloproliferative disorders, and myelodysplastic diseases was originally based on cellular morphology and cytochemistry (immunophenotyping was later added). Using FAB criteria, acute leukemia was defined as greater than 30% bone marrow blasts. The diagnostic criteria used by the World Health Organization (WHO) includes morphologic, cytochemical, immunologic, cytogenetic, and molecular features, as well as clinical findings, to better characterize all hematologic malignancies (myeloid and lymphoid) and predict disease course. The WHO classification defines acute leukemia as the presence of 20% or more bone marrow blasts and includes diagnostic categories with recurrent cytogenetic abnormalities. According to the WHO classification, lymphoid disorders are grouped into B cell, T/NK cell, and Hodgkin lymphoma. Further division of the B and T cell neoplasms considers site of involvement and precursor cell versus mature cell conditions.

198.

A. The blood profile of both chronic myelogenous leukemia (CML) and a neutrophilic leukemoid reaction is characterized by extremely high leukocyte counts with immature neutrophils. Splenomegaly is a manifestation of the malignant disease process and associated with CML rather than a leukemoid reaction. The presence of toxic granules and Döhle bodies would be typical of a leukemoid reaction caused by a severe bacterial infection. The LAP score is low in CML and high in a neutrophilic leukemoid reaction.

199.

C. In Color Plate 17■, the malignant blast cell contains an Auer rod, composed of fused primary granules, which stains positive with both myeloperoxidase and Sudan black B. Auer rods are not seen in lymphoblasts, and their presence can be diagnostic of acute myelogenous leukemia, such as acute myeloblastic leukemia (FAB types M1 and M2) or acute myelomonocytic leukemia (FAB type M4). Multiple Auer rods may be seen in acute promyelocytic leukemia (FAB type M3). Auer rods stain negatively with LAP, which detects the enzyme alkaline phosphatase in neutrophil granules.

200.

D. At presentation, patients with chronic leukemia (e.g., CLL or CML) consistently have elevated leukocyte counts, whereas individuals with acute leukemia may present with low, normal, or high leukocyte counts. The hallmark findings of anemia, thrombocytopenia, and neutropenia are often found in patients with acute leukemia at the time of diagnosis and are due to replacement of normal marrow hematopoietic cells by blasts. Patients with chronic leukemia may have few symptoms at onset, with anemia and thrombocytopenia developing during progression of the disease.

201.

D. Leukocyte alkaline phosphatase (LAP) scores are usually low in patients with chronic myelogenous leukemia (CML). The LAP reflects alkaline phosphatase activity in neutrophils, and the score is usually elevated in conditions where neutrophils are activated and/or increased in number, such as late pregnancy, bacterial infection, and polycythemia vera. The primary use of the LAP is to distinguish between the malignant cells of CML and a severe bacterial infection (leukemoid reaction). It may also be used to distinguish between CML and other chronic myeloproliferative disorders such as polycythemia vera. The LAP may be called NAP (neutrophil alkaline phosphatase) stain.

202.

C. Acute viral hepatitis is associated with lymphocytosis. The major causes of neutrophilia are bacterial infection, neoplastic tumors, and inflammatory responses to tissue injury. "Toxic" neutrophils may be present (toxic granulation, Döhle bodies, vacuolization). Infection with organisms other than bacteria (fungi, some parasites, certain viruses) may also cause neutrophilia.

203.

D. Monocytes must be distinguished from reactive lymphocytes, which are the characteristic feature of infectious mononucleosis. Monocytosis occurring in the recovery stage of acute infections is considered a favorable sign. An increase in monocytes is associated with collagen disorders (e.g., rheumatoid arthritis), tuberculosis, and malignant conditions such as myelodysplastic syndromes and monocytic leukemias.

204.

B. The periodic acid–Schiff (PAS) stain can be used to detect intracellular glycogen deposits in the lymphoblasts of acute lymphoblastic leukemia (ALL), in which coarse clumps of PAS positive material may be observed. Myeloblasts and monoblasts usually show a faint staining reaction. The immunophenotype has a much greater diagnostic value for ALL than the cytochemical stain results. The PAS may also be used to distinguish the malignant erythroid precursors of acute erythroleukemia, which show strong PAS positivity, from normal erythrocytic cells that stain negative.

205.

D. Myelodysplastic syndromes (MDSs) are characterized by a hypercellular bone marrow and up to 20% marrow blasts that distinguish MDS from acute leukemia (using WHO criteria). The blood and bone marrow blast percentages differ, and the risk of transformation to acute leukemia varies with the types of MDSs. These disorders are characterized by one or more peripheral blood cytopenias along with features of abnormal growth (dyspoiesis) in the bone marrow. A consistent feature in all types of myelodysplasia is unexplained and refractory anemia. Abnormalities may be morphologic and/or functional. Criteria that help define the types of myelodysplastic syndromes include megaloblastoid maturation of erythroid precursors, presence of multinucleated red cells, ringed sideroblasts, hypogranular and/or hyposegmented neutrophils, monocytosis, abnormal platelet morphology, circulating micromegakaryocytes, and degree of dyspoiesis.

206.

A. Naphthol AS-D chloroacetate esterase (specific) reacts strongly in granulocytic cells, and alpha-naphthyl acetate esterase (nonspecific) stains positively in monocytic cells. The esterase stains are used to distinguish between subtypes of acute myelogenous leukemia. The cells of acute myeloblastic leukemia (FAB types M1 and M2) will stain positive with specific esterase and negative with nonspecific esterase. The cells of acute monocytic leukemia (FAB type M5) will stain positive with nonspecific esterase and negative with specific esterase. The cells of acute myelomonocytic leukemia (FAB type M4) will show positivity with both specific and nonspecific esterase. Stain results are correlated with cell morphology, immunophenotype, and karyotype for diagnosis.

207.

A. May-Hegglin anomaly is an autosomal dominant disorder in which large blue cytoplasmic structures that resemble Döhle bodies are found in the granulocytes and possibly the monocytes. Leukocytes are normal in function. Platelets are decreased in number and abnormally large. About one-third of patients have mild to severe bleeding problems because of abnormal platelet function.

208.

D. Alder-Reilly anomaly is a hereditary autosomal recessive disorder caused by a deficiency of enzymes involved in the metabolism of mucopolysaccharides. Partially degraded mucopolysaccharides accumulate in various tissues, organs, and the leukocytes that are characterized by the presence of large azurophilic granules resembling toxic granulation. The inclusions do not affect leukocyte function and are referred to as Alder-Reilly bodies. The anomaly is often associated with facial and skeletal abnormalities, such as those seen in Hunter syndrome and Hurler syndrome. Lysosomal fusion with impaired degranulation is the defect in Chédiak-Higashi syndrome and is associated with early death due to abnormal leukocyte function.

209.

C. Serum and urine protein electrophoresis detects the presence of an M spike, the first essential step in establishing the disorder as a monoclonal gammopathy such as multiple myeloma or Waldenström disease. This can be followed by immunoelectrophoresis to determine the class of immunoglobulin or chain type. Immunologic markers, cytochemical stains, and/or cytogenetics are used in conjunction with cell morphology to diagnose malignant conditions.

210.

C. The prognosis is poor for patients with stage IV Hodgkin disease, in which there is widespread disease including bone marrow involvement. Stage I or II Hodgkin disease has a very good prognosis for cure. The clinical course and treatment varies with the extent of disease and morphologic subtype (Rye classification). The peak incidence for Hodgkin lymphoma occurs in young adults (late twenties). Men have a 50% higher incidence of the disease than women. The CRP level and ESR are increased during active disease and can be used to monitor remission status.

211.

C. The acute leukemia indicated by these results is acute myelomonocytic leukemia (AMML), which has both granulocytic and monocytic features. Note the monocytic characteristics of the blast cells in Color Plate 18■. CD14 is a monocytic marker and CD33 is a marker for primitive myeloid cells. The SBB shows positive staining in both granulocytic and monocytic cells, the specific esterase stains positive in granulocytic cells, and the nonspecific esterase is positive in monocytic cells.

212.

B. Acute lymphoblastic leukemia (ALL) of children has the best prognosis. Other favorable factors include children between ages 3 and 7, mild to moderate increases in the peripheral white blood count prior to treatment, and precursor B ALL, CALLA positive type (rather than T cell ALL). Certain cytogenetic and molecular abnormalities are also associated with a better prognosis. Acute leukemia in adults is less favorable because remissions are shorter and more difficult to induce, especially in those over 70 years of age. Prognosis is poor in adults with ALL.

213.

B. The test that would be the most beneficial for the diagnosis of Hodgkin lymphoma is a lymph node biopsy. Lymphadenopathy is the major clinical presentation of Hodgkin disease, and early stages do not have bone marrow involvement. A skin biopsy would be indicated for diagnosis of mycosis fungoides, a T cell lymphoma of the skin. A bone marrow exam and spinal tap are important to the diagnosis of acute leukemias.

214.

A. A hypercellular bone marrow and high M:E ratio are most characteristic of the excessive granulocyte production that occurs in chronic myelogenous leukemia. Polycythemia vera typically has a hypercellular marrow with panhyperplasia and a normal or low M:E ratio. Beta-thalassemia major is a severe hemolytic anemia in which RBC hyperplasia of the marrow is pronounced and a low M:E ratio is usual. Aplastic anemia is associated with a hypocellular marrow with a reduction of all cell lines and normal M:E ratio.

215.

B. Refractory anemia with ringed sideroblasts (RARS) is a myelodysplastic syndrome (MDS) that may also be referred to as primary or idiopathic sideroblastic anemia. The main findings that characterize this type of MDS include refractory anemia with a heterogeneous population of red cells, a hypercellular bone marrow with $<5\%$ blasts, and the presence of $>15\%$ ringed sideroblasts in the marrow (demonstrated with Prussian blue stain). RA and RARS are the least likely MDS types to progress to acute myelogenous leukemia.

216.

C. Recent strenuous exercise or other physical and emotional stimuli cause a transient increase in the leukocyte count. This is due to a redistribution of the blood pools. Marrow injury to stem cells or marrow replacement by malignant cells causes neutropenia of varying degrees. Neutropenia may be caused by immune mechanisms (antibodies) or an overactive spleen that sequesters neutrophils. Chemotherapeutic drugs also suppress bone marrow production of neutrophils.

217.

C. Primary polycythemia (vera) is a malignant myeloproliferative disorder characterized by autonomous marrow production of erythrocytes in the presence of low erythropoietin levels. Usual findings include increased RBC mass with elevated hemoglobin values and variable degrees of leukocytosis and thrombocytosis (pancytosis). Splenomegaly, a high LAP score, thrombotic tendencies, and problems caused by blood viscosity are typical. Phlebotomy is done to reduce red cell mass.

218.

D. Basophilia (and eosinophilia) is a typical finding in patients with chronic myelogenous leukemia (CML). A progressive increase in basophil number suggests transformation of the disease to a more accelerated phase. Myeloproliferative disorders such as CML, polycythemia vera, or AML are often associated with peripheral basophilia, which is not a feature of lymphoproliferative disorders such as acute lymphoblastic leukemia, hairy cell leukemia, or plasma cell leukemia.

219.

C. More than 50% of the marrow cells are erythroid in acute erythroleukemia (FAB type M6). Giant erythroid precursors, bizarre and multinucleated red cells, and increased myeloblasts are found in the marrow and may appear in the blood. Acute erythroid leukemia is rare, and the disease typically evolves into acute myeloblastic leukemia (FAB types M1 or M2).

220.

D. Plasma cell myeloma is a clonal disease involving malignant end-stage B cells, in which overproduction of immunoglobulin is a hallmark and the presence of red cell rouleaux is a characteristic finding on the blood smear, as shown in Color Plate 20■. Excessive amounts of a monoclonal immunoglobulin result in the deposition of proteins on circulating red cells that causes red cell "coining." The erythrocyte sedimentation rate is extremely elevated because of spontaneous rouleaux formation. Multiple myeloma is characterized by bone pain and spontaneous bone fractures caused by tumors of plasma cells. Bone destruction leads to elevated calcium levels, and renal impairment can result from damage by excess light chains. Plasma cells progressively crowd out normal bone marrow precursors and may be found in the blood circulation in advanced disease. Treatment with thalidomide has improved survival.

221.

C. The production of hematopoietic cells in sites outside of the bone marrow can be referred to as myeloid metaplasia or extramedullary hematopoiesis. Hematopoiesis, with the exception of lymphopoiesis, is normally confined to the bone marrow during postnatal life. Production of erythroid, myeloid, and megakaryocytic elements can be established in the liver and spleen, similar to that which occurs during embryonic development. Myeloid metaplasia is frequently associated with myelofibrosis, a condition in which the marrow is gradually replaced by fibrotic tissue.

222.

A. Prominent lymphadenopathy is the most consistent finding in non-Hodgkin types of lymphoma at presentation, but lymphoma may also arise in the spleen, liver, or GI tract (abdominal tumor). Lymphomas begin as localized tumors involving lymphoid tissue that spread to the bone marrow and blood (depends on type). The malignant lymphoid cells are immunologically classified as B cell (most common) or T/NK cell. Clonality can also be established by demonstrating gene rearrangements via molecular analysis. Some common subtypes of non-Hodgkin lymphoma are small lymphocytic, Burkitt, follicular, and mantle cell lymphomas. Leukemias are initially systemic disorders primarily involving the bone marrow and blood at onset. Bone lesions are associated with multiple myeloma.

Methodology

223.

B. The standard assay for hemoglobin utilizes potassium ferricyanide. This solution, formerly called Drabkin's reagent, is now called cyanmethemoglobin (HiCN) reagent. The ferricyanide oxidizes hemoglobin iron from ferrous (Fe^{2+}) to ferric (Fe^{3+}), and the potassium cyanide stabilizes the pigment as cyanmethemoglobin for spectrophotometric measurement.

224.

B. The band containing hemoglobin A_2 is the slowest-migrating, staying closest to the cathode. The band containing hemoglobin A has a net negative charge at an alkaline pH, and it moves the farthest toward the anode. An adult patient without a hemoglobinopathy will have only these two bands appearing on a cellulose acetate electrophoresis.

225.

C. Hemoglobins S, D, and G all migrate to the same location on the hemoglobin electrophoresis gel at an alkaline pH. However, because hemoglobins D and G are nonsickling hemoglobins, tests based on sickle formation under decreased oxygen tension will have negative results. These hemoglobins can be further differentiated by their movement on agar gel at an acid pH, whereas hemoglobins D and G will migrate with hemoglobin A, not with hemoglobin S.

226.

C. When the sample is deoxygenated, reduced hemoglobin S polymerizes, resulting in a cloudy solution. A false negative can be obtained if the quantity of hemoglobin S is below the sensitivity of the method, which can be seen in newborns and anemic patients. Although this procedure is a screening test for hemoglobin S detection, it is positive in the presence of any sickling hemoglobin, such as hemoglobin C_{Harlem}.

227.

A. A slanted column increases the ESR. A clotted sample, which lacks fibrinogen, causes a falsely decreased ESR. Fibrinogen is the plasma protein that most greatly affects the ESR. The EDTA tube for ESR must be at least half-full, and the test must be set up within 4 hours of draw; failure to follow these guidelines results in poikilocytosis that will inhibit rouleaux formation.

228.

C. Some patients develop EDTA-dependent platelet agglutinins caused by an IgM or IgG platelet-specific antibody. To correct for this, the sample can be redrawn in sodium citrate and rerun. The dilution factor of blood to anticoagulant in sodium citrate is 9:1. To compensate for the 10% dilutional loss of platelets, the platelet count obtained must be multiplied by 1.1 ($300 \times 10^9/L \times 1.1 = 330 \times 10^9/L$).

229.

B. Blood smears should be made within 5 hours of collection from blood anticoagulated with EDTA. Although some of the blood cells may still be normal in blood kept longer, others (especially granulocytes) may deteriorate. Vacuolation of neutrophils can appear as an artifact in blood kept past this time. The age of the blood may also affect the visual quality when the slide is stained.

230.

A. Decreasing the angle will produce a longer, thinner smear. Increasing the angle or using a smaller drop of blood will produce a shorter, thicker smear. The angle normally used for the spreader slide when making a smear is 30–45 degrees.

231.

D. One type of Romanowsky stain is the Wright's stain. It is a polychrome stain consisting of methylene blue and eosin. This combination causes multiple colors to appear on staining. Another commonly used Romanowsky stain is the Wright's-Giemsa stain. Brilliant green and neutral red are used in a supravital stain for Heinz bodies. Crystal violet and safranin are used in Gram's stain for bacteria.

232.

C. When red blood cells are stained correctly with Wright's stain, their color is pink to orange-red. They will appear bright red in the presence of an acid buffer and stain. Staining elements such as white cells, which stain with a more basic pH, will not take up the stain adequately in this instance. Inadequate washing and an alkaline stain or buffer mixture results in a smear that is excessively blue.

233.

C. The formula for calculating a reticulocyte count in percent is

$$\frac{\text{Number of reticulocytes counted}}{\text{Total number of RBCs counted}} \times 100$$

In the case described in question 233,

$$\%\text{Reticulocytes} = \frac{60}{1000} \times 100 = 6.0\%$$

Because the error in reticulocyte counts is high, it is desirable to count a larger number of cells or use a standardized counting method such as the Miller disk.

234.

C. The formula used to calculate the absolute reticulocyte count is

$$\frac{\text{Reticulocyte percent}}{100} \times \text{RBC } (10^{12}/\text{L}) \times 1000$$

Multiplication by 1000 is done to report the results in SI units of 10^9/L.

In this case, $\frac{6.0 \times 3.00}{100} \times 1000 = 180 \times 10^9/\text{L}$

$180 \times 10^3/\mu\text{L}$ is not expressed in SI units.

235.

B. As visualized in Color Plate 19■, Sudan black B is a cytochemical stain for lipids, including steroids, phospholipids, and neutral fats. It is widely used as a tool to differentiate the blasts of acute lymphoblastic leukemia (ALL) from those of acute myelogenous leukemia (AML). Blasts in ALL are SBB negative, whereas those in AML will show some degree of positivity.

236.

C. An LAP score is determined by first multiplying the number of cells found by the degree of positivity (i.e., $20 \times 1 = 20$). These numbers are then added together to obtain a final score. In this instance, $0 + 20 + 60 + 60 + 60 = 200$.

237.

C. When stained with a mixture of potassium ferricyanide and hydrochloric acid, nonheme iron stains bright blue. This is the most common stain used for storage iron. It can be used on bone marrow to identify sideroblasts, peripheral blood to identify the presence of siderocytes, or urine to perform hemosiderin testing.

238.

C. Pappenheimer bodies are iron deposits associated with mitochondria, and they stain with both Perl's Prussian blue and Wright's stain. A cell that contains Pappenheimer bodies is called a siderocyte. Howell-Jolly bodies and basophilic stippling can be visualized with Wright's stain, whereas Heinz bodies require a supravital stain to be seen.

239.

B. Depth on a standard counting chamber is 0.10 mm. The formula to calculate volume is $V = A \times D$, where V is volume, A is area, and D is depth. When the counting chamber is used, the area may change, depending on the number of ruled squares counted, but the depth remains constant.

240.

B. The standard formula for hemacytometer counts expressed in mm^3 is

$$\frac{\text{Total number cells counted} \times \text{dilution factor}}{\text{Area counted} \times \text{depth}}$$

In this instance,

$$\frac{308 \times 20}{8\ \text{mm}^2 \times 0.10\ \text{mm}} = \frac{6160}{0.8\ \text{mm}^3} = 7700/\text{mm}^3$$
$$= 7.7 \times 10^9/\text{L}$$

241.

B. The Rule of Three states that RBC $\times$ 3 = Hgb and Hgb $\times$ 3 = Hct ± 3 in error-free results. These rules apply only for normocytic, normochromic erythrocytes. One check to determine if an error has occurred is to determine the MCHC. An MCHC should be less than 37.0 g/dL in error-free results. The MCHC is calculated by dividing hemoglobin by hematocrit and multiplying by 100. In instance (B), the MCHC is 38.3 g/dL and the Rule of Three is broken. All other answers follow the Rule of Three.

242.

A. The only *true* cause of a high MCHC is the presence of spherocytes, as may be seen in hereditary spherocytosis. Because the MCHC is a calculation using the hemoglobin and hematocrit, anything causing those parameters to be wrong will affect the MCHC. The occurrence of a falsely high MCHC is much more common than the presence of spherocytes, and specimen troubleshooting procedures must be undertaken to obtain reportable results.

243.

C. Blood cells are nonconductors of electrical current; they create a resistance/impedance of current in a diluent solution that is conductive. When the suspension is forced through a small aperture, the current flow is interrupted by the presence of the cells. A pulse is generated. The number of pulses generated is proportional to the number of particles present, and the size of the pulse generated is proportional to the size of the cell.

244.

C. Using $\pm 2\ s$, 95% confidence limits are achieved; 95% confidence limits predict a range that values should fall within 95% of the time. For example, if a WBC count is $12.0 \times 10^9/L$ with $2\ s$ of ± 0.5, then a succeeding count must be less than $11.5 \times 10^9/L$ or greater than $12.5 \times 10^9/L$ to be considered significantly different.

245.

B. Side angle scatter of a laser beam increases with granularity of the cytoplasm. Forward angle scatter is used to determine relative size. The number of signals is proportional to the number of cells. The presence of specific antigens in the cytoplasm or on the cell surface is determined by immunofluorescence after reactions with appropriate antibodies.

246.

A. An impedance counter cannot differentiate between the nucleus of a white blood cell and the nucleus of an nRBC. Both will be counted as WBCs. The presence of 5 or more nRBCs/100 WBCs can result in a falsely elevated white blood cell count, and a correction must be made as follows:

Corrected WBC count =

$$\text{Observed count} \times \frac{100}{100 + \#\ \text{nRBCs}/100\ \text{WBCs}}$$

247.

D. Hemoglobin A_2 values up to 3.5% are considered normal. Values between 3.5 and 8.0% are indicative of beta-thalassemia minor. Hemoglobins C, E, and O have net electrical charges similar to hemoglobin A_2. They elute off with hemoglobin A_2 using anion exchange (column) chromatography, causing an invalid hemoglobin A_2 result. If the hemoglobin A_2 quantification using column chromatography yields a result greater than 8.0%, one of these interfering hemoglobins should be considered.

248.

A. Any condition with spherocytes can cause an increased osmotic fragility, dependent on the number of spherocytes present. Spherocytes are seen in hereditary spherocytosis, immune hemolytic anemias, and severe burns. Target cells, associated with thalassemias and hemoglobinopathies, have an increased surface area-to-volume ratio and a decreased osmotic fragility.

249.

B. The solubility test for hemoglobin S is not quantitative; it is reported as positive or negative. A clotted specimen will not affect the result. A clotted specimen will falsely decrease the other tests listed: ESR due to low fibrinogen, hematocrit due to a false low RBC count, and platelets are trapped in the clot.

250.

A. Hematology reference intervals are available in many textbooks. They are influenced by patient population, instrumentation, and reagents used. Therefore, it is ideal for each laboratory to establish its own reference intervals. The reference interval excludes the upper and lower 2.5% of the values. The remaining 95% represent the reference interval.

251.

C. Standards are commercially available to generate a hemoglobin concentration curve. The absorbance of each solution is read against a reagent blank at 540 nm on a spectrophotometer. Patient blood samples and commercial control materials can be used to assess precision and other quality control parameters.

252.

A. Anything that causes an increase in absorbance will cause a hemoglobin that is read spectrophotometrically to be falsely high. It is necessary to correct for this type of error, such as making a plasma blank in the case of lipemia or icterus. WBCs are present in the hemoglobin dilution and usually do not interfere. When the WBC count is extremely high, their presence will cause cloudiness, increasing the absorbance in the hemoglobin measuring cell and resulting in a falsely high hemoglobin concentration. Excessive anticoagulant does not affect hemoglobin readings.

253.

A. When a microhematocrit is spun at 10,000–15,000 × *g* for 5 minutes, maximum packing is achieved. Spinning a longer time has no affect on the result. A tube that is not full causes RBC shrinkage and a falsely decreased hematocrit. Hemolysis damages the RBC membrane and allows for more packing. Trapped plasma is present when optimal packing is not achieved due to inadequate speed or time of centrifugation, causing a falsely high hematocrit.

254.

C. The erythrocyte sedimentation rate (ESR) measures the rate of fall of red cells through plasma. ESR increases when cells become stacked (rouleaux, as seen in Color Plate 20■). ESR decreases when cells are not normal discocytes. Larger cells (macrocytes) and fewer cells (anemia) fall faster. Plasma containing increased proteins, such as fibrinogen and globulins, promote rouleaux formation and an elevated ESR. Hemoglobin content does not affect the ESR.

255.

A. Impedance counters measure RBCs and platelets using the same dilution. To differentiate the two, sizing thresholds are used. Particles between 2 and 20 fL are counted as platelets, and particles larger than 35 fL are counted as RBCs. Small RBCs, clumped platelets, and giant platelets fall in the overlap area between platelets and RBCs, generating a warning flag. Nucleated RBCs are larger than normal RBCs and are not mistaken for platelets.

256.

C. A platelet estimate is obtained by multiplying the average number of platelets per oil immersion field (in an erythrocyte monolayer) by 20,000. The reference range for a platelet count is 150–450 × 10^9/L. Approximately 8–20 platelets per oil immersion field will represent a normal platelet concentration of approximately 160–400 × 10^9/L. This method assumes the red blood cell count is normal. If it is not, alternate platelet estimate procedures may need to be performed.

257.

D. A living cell stain using new methylene blue is performed for reticulocyte counts. Reticulocytes should not be stained for less than 5 minutes. Howell-Jolly bodies, Pappenheimer bodies, crenated cells, and refractile artifact can be mistaken for reticulocyte inclusions. Two or more particles of reticulum constitute a reticulocyte.

258.

B. All accredited laboratories are required to perform calibration with commercially available calibrators at least once every 6 months. Calibration must be checked if any major part is replaced or if optical alignment is adjusted. A calibration procedure can be verified using commercially available controls.

259.

A. An MCHC >37.0 g/dL is most likely caused by an error in measurement. In this instance, the Rule of Three shows that the RBC × 9 matches the hematocrit, but the RBC × 3 does not match the hemoglobin. The hemoglobin does not match either the RBC or hematocrit. This indicates a hemoglobin problem, and it can be corrected with a saline replacement procedure. This specimen may be lipemic or icteric. Warming the specimen is useful in troubleshooting a high MCHC due to a cold agglutinin. A microhematocrit would be indicated if the hematocrit result was invalid.

260.

A. Hemoglobin is valid on a hemolyzed specimen, because RBC lysis is the first step in the cyanmethemoglobin method. The red blood cell count depends on the presence of intact red blood cells. Red blood cell fragments caused by hemolysis may be as small as platelets and affect instruments that use sizing criteria to differentiate the two. Therefore, samples for these procedures should be re-collected.

261.

D. Heparin is recommended for osmotic fragility and red cell enzyme studies, because it results in less lysis and less membrane stress than other anticoagulants. Heparin induces platelet clumping and is unacceptable for the platelet count. Heparin is unacceptable for coagulation test procedures because it binds with antithrombin to neutralize many enzymes, especially thrombin. This would cause very long coagulation test results. EDTA is recommended for most routine hematology procedures, especially for Wright's-stained smears. Sodium citrate or EDTA can be used for sedimentation rates.

262.

A. When counting platelets, the center square (1 mm^2) is counted on each side of the hemacytometer. Platelets appear round or oval and may have dendrites. These characteristics can help distinguish them from debris, which is irregularly shaped and often refractile. White cells are not lysed; they may be counted, using a different ruled area of the hemacytometer. Platelets will be easier to count if allowed to settle for 10 minutes, because they will have settled into one plane of focus.

263.

B. "Precision" is the term used to describe the reproducibility of a method that gives closely similar results when one sample is run multiple times. An accurate method is one that gives results that are very close to the true value. Laboratories must have procedures that are both accurate and precise.

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264.

C. WBC counts done by an impedance cell counter must be corrected when nucleated red blood cells (nRBCs) are present (see Color Plate 4■), because such instruments do not distinguish between white and red nucleated cells. This correction is done according to the following formula:

Corrected WBC count =

$$\text{Observed count} \times \frac{100}{100 + \#\ \text{nRBCs per 100 WBCs}}$$

In this instance,

$$35.0 \times \frac{100}{100 + 110} = 16.7 \times 10^9/\text{L}$$

265.

A. The appearance of red cells on a differential smear may be predicted by calculating the red cell indices.

$$\text{MCV} = \frac{\text{Hct} \times 10}{\text{RBC}} = \frac{16\% \times 10}{2.50 \times 10^{12}/\text{L}} = 64.0\ \text{fL}$$

$$\text{MCH} = \frac{\text{Hgb} \times 10}{\text{RBC}} = \frac{4.5\ \text{g/dL} \times 10}{2.50 \times 10^{12}/\text{L}} = 18.0\ \text{pg}$$

$$\text{MCHC} = \frac{\text{Hgb} \times 100}{\text{Hct}} = \frac{4.5\ \text{g/dL} \times 100}{16\%}$$
$$= 28.1\ \text{g/dL}\ (281\ \text{g/L})$$

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are all below the reference range. This indicates a cell that is small (microcytic) with a reduced hemoglobin concentration (hypochromic). These indices refer to averages and do not necessarily reflect the actual appearance of cells in which there is great diversity in size and shape.

266.

D. Children with beta-thalassemia major, also known as Cooley anemia, do not use iron effectively to make heme. This occurs because of a genetic defect that causes a decreased rate of production of structurally normal globin chains. In addition, these children receive frequent transfusions due to the severe hemolytic anemia. The result is hemochromatosis with a high serum iron and storage iron. None of the other anemias listed would elicit the bone marrow response seen by the high number of nucleated RBCs, because they are not hemolytic.

267.

D. Beta-thalassemia major is characterized by an inability to produce beta-globin chains, resulting in a decrease or complete absence of hemoglobin A. Hemoglobin F, a compensatory hemoglobin that contains two alpha- and two gamma-globin chains, is frequently the only hemoglobin present. Hemoglobin A_2 is classically increased in heterozygous beta-thalassemia, but it is variable in homozygous beta-thalassemia.

268.

B. The predominant hemoglobin present at birth is hemoglobin F, which consists of two alpha- and two gamma-globin chains. It is not until about 6 months of age that beta-chain production is at its peak. At this point, hemoglobin A (two alpha and two beta chains) replaces hemoglobin F as the predominant hemoglobin. A deficiency in the production of these chains will not be apparent until this beta-gamma switch has occurred.

269.

D. The formula for calculation of transferrin saturation is as follows:

$$\text{Transferrin saturation } \% = \frac{\text{Serum iron } (\mu g/dL) \times 100}{\text{TIBC } (\mu g/dL)}$$

In this case,

$$\text{Transferrin saturation } \% = \frac{22 \times 100}{150} = 15\%$$

Because the reference range for saturation is 20–45%, this is a low saturation.

270.

D. In the anemia of chronic disease, patients have iron but are unable to utilize it. Hepcidin, a hormone produced by the liver, plays a role in body iron regulation. Intestinal iron absorption and release of iron from macrophages both decrease in response to increased hepcidin levels. Hepcidin is a positive acute-phase reactant, so increased levels are seen in anemia of chronic disease due to inflammation. This adversely affects iron availability.

271.

A. The most common anemia among hospitalized patients is anemia of chronic disease. Patients with chronic infections, inflammatory disorders, and neoplastic disorders develop this type of anemia. The typical presentation is a normocytic, normochromic anemia, but microcytic and hypochromic anemia can develop in long-standing cases. Chronic blood loss can cause iron deficiency and microcytic/hypochromic anemia.

272.

A. Both megaloblastic anemias and some nonmegaloblastic anemias are characterized by the presence of macrocytic, normochromic red cells. Vitamin B_{12} and folic acid are coenzymes necessary for DNA synthesis. Lack of either one causes megaloblastic anemia. Maturation asynchrony is evident in both the peripheral blood and bone marrow. The bone marrow examination is done after vitamin B_{12} and folate levels because of the test's invasive nature. Vitamin B_{12} and folic acid levels are normal or increased in nonmegaloblastic anemias. Iron studies are useful in the diagnosis of microcytic/hypochromic anemias.

273.

C. The common causes of megaloblastic anemia are pernicious anemia and folic acid deficiency. Neurological symptoms are not associated with folic acid deficiency. Folic acid is a water-soluble vitamin for which there are low body stores. A diet low in green vegetables and meat products or high in alcohol can result in folate deficiency in 2–4 months. Alcohol is a folate antagonist.

274.

A. The general classification of anemia described here is megaloblastic anemia. A deficiency of vitamin B_{12} or folic acid affects DNA production. All dividing cells will show nuclear abnormalities, resulting in megaloblastic changes. In the neutrophil, as seen in Color Plate 11■, this takes the form of hypersegmentation (five lobes or more). Enlarged, fragile cells are formed, many of which die in the bone marrow. This destruction leads to increased LD, bilirubin, and iron levels. Oval macrocytes and teardrop cells are seen. Pancytopenia and inclusions are common findings. One cause of a nonmegaloblastic macrocytic anemia, which has round cells such as target cells instead of oval cells, is liver disease.

275.

B. The pancytopenia and red blood cell morphologic findings are all consistent with megaloblastic anemia. Further investigation of serum folate and vitamin B_{12} levels is warranted. Pernicious anemia (PA) is noted for neurological complications and is seen more commonly among people of British and Scandinavian ancestry. PA is caused by a lack of intrinsic factor production in the stomach, which is necessary for the absorption of vitamin B_{12}. Because there are large body stores of vitamin B_{12}, it takes from 1 to 4 years for the deficiency to manifest itself. Aplastic anemia is also associated with pancytopenia, but not the red cell morphologic changes seen in this patient.

276.

A. Alcohol is a folic acid, not a vitamin B_{12}, antagonist. Patients with pernicious anemia (PA) are incapable of absorbing vitamin B_{12} due to a lack of intrinsic factor or antibodies to intrinsic factor or parietal cells. PA is characterized by achlorhydria and atrophy of gastric parietal cells that secrete intrinsic factor. Achlorhydria is not diagnostic for PA, because it may occur in other disorders (such as severe iron deficiency), but it is confirmatory evidence of the problem. *D. latum* competes for B_{12} in the intestines.

277.

B. Because intrinsic factor is necessary for absorption of vitamin B_{12} from the ileum, intramuscular injections of vitamin B_{12} are used to treat PA. Although oral doses of folic acid will correct the megaloblastic blood profile seen in PA, the neurological symptoms will not improve. For this reason, correct diagnosis is crucial. Methotrexate is a folic acid antagonist. Because body folic acid stores are low, a deficiency can develop quickly.

278.

A. The adult red blood cell in glucose-6-phosphate dehydrogenase (G6PD) deficiency is susceptible to destruction by oxidizing drugs. This occurs because the mechanism for providing reduced glutathione, which keeps hemoglobin in the reduced state, is defective. The anti-malarial drug primaquine is one of the best-known drugs that may precipitate a hemolytic episode. Ingestion of fava beans can also elicit a hemolytic episode in some patients.

279.

C. G6PD deficiency, a sex-linked disorder, is the most common enzyme deficiency in the hexose monophosphate shunt. Most patients are asymptomatic and go through life being unaware of the deficiency unless oxidatively challenged. Pyruvate kinase, an enzyme in the Embden-Meyerhof pathway, is necessary to generate ATP. ATP is needed for red blood cell membrane maintenance. Patients with a pyruvate kinase deficiency have a chronic mild to moderate anemia.

280.

B. Reduced glutathione levels are not maintained due to a decrease in NADPH production. Methemoglobin (Fe^{3+}) accumulates and denatures in the form of Heinz bodies. Heinz bodies cause rigidity of the RBC membrane, resulting in red cell lysis. Döhle bodies are composed of RNA; Howell-Jolly bodies are composed of DNA; Pappenheimer bodies are iron deposits.

281.

C. Iron-deficiency anemia (IDA) causes a microcytic, hypochromic anemia. It is the most common anemia found in children. IDA develops quickly in children because of rapid growth with increased dietary iron requirements. Hereditary spherocytosis results in RBCs that are normal to low-normal in size, with an MCHC possibly greater than 37.0 g/dL. Folic acid deficiency causes a macrocytic/normochromic anemia. Erythroblastosis fetalis is a hemolytic disease of the newborn caused by red blood cell destruction by antibodies from the mother; such antibodies are no longer in the circulation of a 15-month-old child.

282.

C. The development of iron deficiency occurs in stages: the iron depletion stage, the iron-deficient erythropoiesis stage, and the iron-deficiency anemia stage. Iron stores are the first to disappear, so the serum ferritin level is the earliest indicator of iron-deficiency anemia. This is followed by decreased serum iron and increased TIBC. The last abnormality seen is microcytic, hypochromic red blood cells.

283.

C. In iron-deficiency anemia, red blood cell production is restricted because of lack of iron, and the reticulocyte absolute value reflects this ineffective erythropoiesis. The formula used to calculate the absolute reticulocyte count is

$$\text{Absolute reticulocytes} = \frac{\text{Reticulocytes }\%}{100} \times \text{RBC } (10^{12}/\text{L}) \times 1000$$

The 1000 in the calculation is to convert to SI units (10^9/L).
In this case,

$$\frac{0.2}{100} \times 2.70\ (10^{12}/\text{L}) \times 1000 = 5 \times 10^9/\text{L}$$

The reference interval for the absolute reticulocyte count is approximately $18–158 \times 10^9$/L.

284.

A. Petechiae and ecchymoses (bruises) are primary hemostasis bleeding symptoms seen in quantitative and qualitative platelet disorders. Although estimates vary, spontaneous bleeding does not usually occur until platelet numbers are less than 50×10^9/L. The malignant disorder represented in this case is noted for thrombocytopenia.

285.

A. The bone marrow blast percent indicates the presence of an acute leukemia. The triad of symptoms seen in acute leukemia is neutropenia, anemia, and thrombocytopenia. Acute lymphoblastic leukemia is the leukemia most likely to be found in this age group. Hairy cell leukemia does not present with blasts. Myelodysplastic syndrome presents with less than 20/30 (WHO/FAB, respectively) percent marrow blasts.

286.

C. Periodic acid–Schiff stains glycogen in lymphoblasts. The myeloperoxidase stain is positive in myeloid cells. Monocytes show a positive reaction to the nonspecific esterase stain. Leukocyte alkaline phosphatase is useful in the diagnosis of chronic myelogenous leukemia.

287.

A. Terminal deoxyribonucleotidyl transferase (TdT) is a nuclear enzyme (DNA polymerase) found in stem cells and precursor B and T lymphoid cells. High levels of TdT are found in 90% of ALLs. TdT has been found in up to 10% of cases of AML (FAB M0 and M1), but in lower levels than are present in ALL. This enzyme is not found in mature lymphocytes.

288.

B. There are now more than 200 recognized human leukocyte antigens, each of which has been given a CD (cluster designation) number. CDs 2, 5, and 7 are seen on T cells. CALLA, the common acute lymphoblastic leukemia antigen, is seen in early pre–B cells. Distinct CD markers have been identified for cells of both lymphoid and myeloid stem cell lineage.

289.

B. The bone marrow blast percent is high enough to indicate an acute leukemia. Sudan black B, myeloperoxidase, and specific esterase stains are positive, indicating the presence of the myeloid cell line. The nonspecific esterase stain is negative, indicating the absence of a monocytic cell line. The bone marrow blast percent is too low for FAB M1, but it is in the range for FAB M2.

290.

A. Chromosome analysis is an important diagnostic tool in clinical medicine. Nonrandom chromosome abnormalities are recognized in many forms of cancer. t(8;21) is associated with acute myelogenous leukemia FAB M2; t(15;17) is only seen in FAB M3. The Philadelphia chromosome, t(9;22), is seen in at least 90% of patients with chronic myelogenous leukemia; t(8;14) is associated with Burkitt lymphoma.

291.

B. When a diagnosis of AML or myelodysplastic syndrome is suspected, a bone marrow examination is performed. The WHO approach to the diagnosis of acute leukemia requires the presence of >20% blasts in the bone marrow; the FAB classification requires >30%. The reference interval for bone marrow blast percent is 0–2%. Myelodysplastic syndromes have increased bone marrow blast percentages, but <20% using WHO criteria and <30% using FAB criteria.

292.

B. HTLV-I is implicated in T cell leukemia and lymphoma in Japan. The Epstein-Barr virus is associated with Burkitt lymphoma in Africa. Chronic bone marrow dysfunction can be caused by exposure to radiation, drugs, and chemicals such as benzene. Myelodysplastic syndromes and myeloproliferative disorders are "preleukemic" because they have a high incidence of terminating in acute leukemia. Paroxysmal nocturnal hemoglobinuria, aplastic anemia, multiple myeloma, and lymphoma are stem cell disorders that are particularly noted for transformation into acute leukemia. Genetic susceptibility is associated with Klinefelter and Down syndromes, both of which have chromosomal abnormalities. It is likely that more than one factor is responsible for the evolution of an acute leukemia.

293.

A. The myelodysplastic syndromes (MDSs) are pluripotential stem cell disorders characterized by one or more peripheral blood cytopenias. Bone marrow examination is necessary for diagnosis. There are prominent maturation abnormalities in all three cell lines in the bone marrow. Megaloblastoid erythrocyte maturation is present that is not responsive to B_{12} or folic acid therapies. Although many of the red blood cell inclusions noted in this case can be seen in a megaloblastic anemia such as pernicious anemia, this patient has a normal vitamin B_{12} and folate level. This patient has hyposegmentation of neutrophils, whereas megaloblastic anemias present with hypersegmentation of neutrophils. Of the disorders listed, the only one associated with dyshematopoiesis of all cell lines is myelodysplastic syndrome.

294.

D. In most cases of MDS, the bone marrow is hypercellular with erythroid hyperplasia. MDS is considered a disease of the elderly. Because normal cellularity decreases with age, interpretation of cellularity must take the age of the patient into account. WHO criteria for a diagnosis of MDS are related to bone marrow blast percent, which must be <20%.

295.

D. In RAEB at least two cell lines exhibit cytopenia, and all cell lines show evidence of dyshematopoiesis. Poor granulation and pseudo-Pelger-Huët anomaly is seen. There are less than 5% blasts in the peripheral blood, and between 5 and 19% blasts in the bone marrow. Platelets exhibit poor granulation, giant forms, and the abnormal maturation stage of micromegakaryocytes. The five FAB classifications of myelodysplastic syndrome are RA, RARS, RAEB, CMML, and RAEB-t. CML is a myeloproliferative disorder, not a myelodysplastic syndrome.

296.

C. The myelodysplastic syndromes are refractory to treatment, and patients are supported using blood products dependant on their cytopenias. The median survival rate for all types of MDSs is less than 2 years. RAEB and RAEB-t have the highest percentage of blasts and the lowest survival rates. At this time, bone marrow transplant offers the only chance for a cure, and it is the treatment of choice in patients less than 50 years old. Studies have shown that the incidence of MDS is greater than the incidence of AML in the 50–70-year-old age group. Up to 40% of the myelodysplastic syndromes transform into acute leukemia.

297.

B. Chronic myelogenous leukemia (CML) is a myeloproliferative disorder, a malignant proliferation of leukocytes not in response to infection; the leukocyte count is often greater than 100.0×10^9/L. No toxic changes are present in CML. Thrombocytosis is seen in more than half of the patients with CML. A neutrophilic leukemoid reaction represents a normal body response to a severe infection. It is a benign proliferation of WBCs with a high leukocyte count but usually less than 50.0×10^9/L. Toxic changes to the neutrophils such as toxic granulation, vacuoles, and Döhle bodies are present. These two disorders both display a "left" shift, and they can be confused with each other.

298.

D. Leukocyte alkaline phosphatase activity is increased in severe infections such as the neutrophilic leukemoid reaction and polycythemia vera, and during the last trimester of pregnancy. It is greatly reduced in chronic myelogenous leukemia, although it may increase during blast crisis of this disease. Periodic acid–Schiff and Sudan black B are used to differentiate ALL from AML. The TRAP stain is useful in the diagnosis of hairy cell leukemia.

299.

C. The Philadelphia chromosome, t(9;22), is found in the precursor cells for erythrocytes, granulocytes, and platelets in at least 90% of the cases of CML. It is an acquired chromosome abnormality that results from a reciprocal translocation between chromosomes 9 and 22, and it can be detected even when the patient is in remission. The BCR/ABL oncogene is also associated with CML.

300.

B. Patients who have the Philadelphia chromosome have a less aggressive disease and better prognosis than the rare cases that do not have the abnormality. Some cases of CML terminate in an acute lymphoblastic leukemia. However, this outcome cannot be predicted by the presence or absence of the Philadelphia chromosome.

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